INTERACTIVE ASSOCIATION BETWEEN MOLECULAR STRUCTURE SPECTRAL PROFILES AND NUTRIENT UTILIZATION AND AVAILABILITY OF LEAF, STEM, PODS, AND WHOLE PLANT FABA BEAN FORAGE IN RUMINANTS BEFORE AND AFTER RUMEN INCUBATION.

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon

By

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ABSTRACT

The general objectives of this study were to: (1) compare structural, physiochemical, and nutritional characterization among faba bean samples (stem, leaf, whole pods, whole plant and whole plant silage) and (2) use different parts of faba bean, whole plant faba bean and whole plant faba bean silage as references to study the effect of rumen digestion on the change of its spectral structure and its association to protein and carbohydrate digestion and metabolism characteristics. The faba bean samples used for this research were from three different plots in Yellow Creek, Saskatchewan with 97 days maturity (late pod stage) and the variety used was CDC Snowdrop. This research consists of three Projects. In Chapter 3, chemical analyses, energy parameters, CNCPS fractions, rumen degradation kinetics, N to energy synchronization, intestinal digestion and metabolizable protein supply prediction with NRC-2001 and DVE/OEB systems were conducted. In Chapter 4, both univariate and multivariate analyses of FTIR spectroscopy were conducted for selected spectral parameters and spectral regions in original and 12 and 24 h incubation residue samples. In Chapter 5, interactive association between nutritional data in Chapter 3 and spectral data in Chapter 4 were studied. Significance was declared at $P < 0.05$ and tendency at $0.05 \leq P \leq 0.10$ in all studies. In Chapter 3, whole pods (WP) of faba bean had the highest starch, crude protein (CP), soluble crude protein (SCP) and the least acid detergent insoluble crude protein (ADICP). Total digestible nutrients (TDN), rumen degradable protein (RUP) intestinal digestibility and feed milk value (FMV) were also highest in whole pods faba bean, therefore it had the highest nutritional values. Whole plant faba silage compared with whole plant (WPL) faba bean had higher ($P < 0.05$) starch and CP contents; and lower ($P < 0.05$) degradation rate (k_d) of NDF, effective digestible NDF (EDNDF). In NRC-2001 system, Leaf, WP and faba silage had comparable ($P > 0.1$) metabolizable protein supply and FMV. But in

DVE/OEB system, WP had the highest metabolizable protein supply and FMV; WPL was only numerically higher than silage in metabolizable protein supply and FMV. In Chapter 4, as for protein related spectral parameters, peak height of beta sheet, amide I, peak area of amide II and area ratio of amide I to amide II, spectral intensity was decreased with increasing time of incubation. In other spectral parameters, incubation time was interacted with different types of faba bean samples. As for carbohydrate related spectral parameters of total carbohydrate first peak (TC1) height and cellulosic compounds (CEC) peak height and peak area, spectral intensity was increased after incubated in the rumen. Faba bean samples significantly interacted with incubation time for other spectral profiles. With multivariate analyses, it is found that (1) Original samples can be discriminate from incubation residue samples; (2) 12 and 24 h incubation samples were grouped together. As a result, spectral features were altered during first 12 h of rumen incubation. In Chapter 5, DVE/OEB model compared with NRC-2001 model had stronger correlation with protein related spectral profiles of faba bean. CP, SCP and acid detergent insoluble crude protein (ADICP) had stronger correlation with original samples spectral profiles, NDICP had stronger correlation with incubation residue spectral profiles. In addition, readily digestible carbohydrate fractions had stronger correlation with original samples spectral profiles. Less degradable and undegradable carbohydrate fraction had stronger correlation with 12 and 24 h rumen incubation residue spectral profiles. Furthermore, DVE, effective degradable crude protein (EDCP), FMV, CNCPS carbohydrate subtractions of CB1, CB2 and CC, non-fiber carbohydrate (NFC), starch and total digestible NFC were precisely predicted (regression coefficient greater than 0.90) using original and degradation residues spectral profiles. In general, carbohydrate and protein related spectral features could be used as indicators for faba bean nutritional evaluation in dairy cattle;

whole plant faba bean, whole pods faba bean and faba silage can be used as potential feed ingredient for dairy cows.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor Dr. Peiqiang Yu of the college of Agriculture and Bioresources at the University of Saskatchewan for providing me the valuable chance to learn the knowledge of ruminant nutrition and feed science in your team. Thank you greatly for your valuable guidance, help and suggestion during the graduate study. Special thanks to Zhiyuan Niu of the department of Animal Science for the detailed laboratory training and support in daily living. Thanks for Dr. Yaogeng Lei for his discussion and suggestion during my learning and what's more important for his inspiration of how to solve problem independently. I also want to express my sincere thanks to my Advisory Committee, Dr. David Christensen, Dr. Herbert (Bart) Lardner, and Dr. Ryan Brook for their academic suggestion, professional comments and friendly encouragement. I also want to thank my fellow students Victor, Maria, Aya, Marcela, Jae, Chaoli, Alessandra and PDF fellows Luciana, Basim and Xin Feng for their suggestion and discussion during my graduate study. I would like to acknowledge the Government of Saskatchewan, Saskatchewan Pulse Growers, Natural Sciences and Engineering Research Council of Canada (NSERC), Ministry of Agriculture Strategic Feed Research Chair (Dr. Peiqiang Yu), and the Rayner Dairy Research and Teaching Facility for the funding and resources to develop and complete this research. Finally but most importantly I would like to thank my parents Bin Yan and Limin Cheng, my grandparents Qinan Yan and Xixiang Chen for their unconditional support for every choice I made and thank my girlfriend Zeyu Qi for all the happiness and companion she brought me.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
ADL	Acid detergent lignin
AMCP	Truly absorbed microbial protein in the small intestine
ARUP	Truly absorbed rumen undegraded protein in the small intestine (NRC Dairy model)
ATR	Attenuated total reflectance
BCP	Rumen bypass feed crude protein (DVE/OEB system)
BDM	Rumen bypass dry matter
BDNDF	Rumen bypass feed neutral detergent fiber
CA4	Rapidly degradable carbohydrate fraction (sugar)
CB1	Intermediately degradable carbohydrate fraction (starch)
CB2	Intermediately degradable carbohydrate fraction (soluble fiber)
CB3	Available neutral detergent fiber or slowly degradable carbohydrate fraction (Digestible fiber)
CC	Unavailable neutral detergent fiber (Indigestible fiber)
CDC	Crop Development Center
CEC	Cellulosic compounds region
CHO	Carbohydrates
CP	Crude protein
CLA	Cluster analysis
D	Degradable fraction
$DE_{p3\times}$	Digestible energy at a production level (3× maintenance)

dIDP	Intestinal digestibility of rumen bypass protein
DM	Dry matter
DPB	Degraded protein balance
DVBE	Truly absorbed bypass feed protein in the small intestine
DVE	Total truly digested protein in the small intestine (DVE/OEB system)
DVME	Truly absorbed rumen synthesized microbial protein in the small intestine
EE	Ether extract (crude fat)
ECP	Rumen endogenous protein
ED_CHO	Effectively degraded carbohydrate
ED_N	Effectively degraded nitrogen
EDCP	Effective degraded crude protein
EDDM	Effective degraded dry matter
EDNDF	Effective degraded neutral detergent fiber
FMV	Feed milk value
FTIR	Fourier transform infrared spectroscopy
uNDF	Undigestible neutral detergent fiber
K_d	Degradation rate of potentially degradable fraction
K_p	Passage rate
MCP_{RDP}	Microbial protein synthesized in the rumen based on rumen degraded protein
MCP_{TDN}	Microbial protein synthesized in the rumen based on available energy (total digestible nutrients at a production level)
DE	Digestible energy
NE	Net energy

ME	Metabolizable energy
$ME_{p3\times}$	Metabolizable energy at a production level (3× maintenance)
MP	Metabolizable protein (NRC Dairy model)
NDF	Neutral detergent fiber
NDICP	Neutral detergent insoluble crude protein
NE_g	Net energy for gain
$NE_{Lp3\times}$	Net energy for lactation at a production level (3× maintenance)
NE_m	Net energy for maintenance
NFC	Non-fiber carbohydrate
NPN	Non-protein nitrogen
OEB	Degraded protein balance (DVE/OEB system)
PA2	Rapidly degradable true protein (soluble true protein)
PB1	Moderately degradable true protein (insoluble true protein)
PB2	Slowly degradable true protein (fiber bound protein)
PC	Indigestible protein
RDNDF	Rumen degradable fiber (NDF)
RDP	Rumen degradable protein
RUDM	Rumen undegradable dry matter
RUNDF	Rumen undegradable neutral detergent fiber
RUP	Rumen undegradable protein
S	Soluble fraction
SR-IMS	Synchrotron radiation infrared microspectroscopy
ST	Structural carbohydrate region

SCP	Soluble crude protein
T0	Lag time
TC	Total carbohydrate region
tdCP	Truly digestible crude protein
TDDM	Total digested dry matter
tdFA	Truly digestible fatty acid
$TDN_{1\times}$	Total digestible nutrients at a maintenance level
TDNDF	Total digested neutral detergent fiber
tdNDF	Truly digestible neutral detergent fiber
tdNFC	Truly digestible non-fiber carbohydrate
TDP	Total digested crude protein
TMR	Total mixed ration
VC	Vicine and convicine
U	Rumen undegradable fraction
WPL	Whole plant of faba bean
WP	Whole pods of faba bean

1. INTRODUCTION

The increasing global population and improving living quality lead to escalating demand of animal protein. However, with the progress of urbanization the land left for agriculture production was shrinking. As a result, the current situation requires agricultural practitioner to make the most efficient use of their land. Alternative feeding resource provides farm owner with more options when making the wisest choice based on consideration of series of aspects like yield, market, and climate. In western Canada, legumes such as dry peas, chickpeas, lentils, and dry beans are widely planted for both human consumption and livestock feedstuff because of the natural advantages of soil and climate. Faba bean as a type of legume with seeds having high starch and protein contents also shows its potential as feedstuff and food resources both domestically and globally (Phelps 2017). As feed resource, the antinutritional factors of tannin and VC are main concern (Phelps 2017), however, proper tannin content is considered beneficial for ruminant which decrease degradation rate of protein (He et al. 2019). Therefore, faba bean have potential to be used as feed resource for ruminant. In recent years, studies about substituting soybean with faba bean seeds in diets of high production dairy cattle has been conducted and acquire desirable results (Cherif et al. 2018; Mendowski et al. 2019). The ensiling potential of faba bean was also tested *in vitro* (Mustafa and Seguin 2003) and is found to be applicable. However, there is no research about the nutritional value of faba bean partitions and whole plant faba bean forage for ruminant.

Nutritionally, feed chemical compositions are partitioned into those readily digested in the rumen and those bonded with cell wall and are slowly or undegradable in the rumen. The readily digestible fractions such as starch, sugars, lipid, and soluble crude protein are more easily to quantify its contribution to ruminant. However, the nutritional value of compounds bonded within cell wall is hard to determine because the complex structure and complicated cross-linkage

between cellulose, hemicellulose, lignin, pectin, and structural protein preventing it from enzymatic hydrolysis (Raffrenato et al. 2017). Cell wall polysaccharide matrix of cellulose, hemicellulose, lignin, pectin and protein has three interwoven polymer networks (Pettolino et al. 2012). Cellulose is formed by β-(1,4) linkage of glucose and several cellulose chains are then aggregated by hydrogen bonds to form microfibrils. The network of cellulose coating with hemicellulose embedded with pectin to form gel-like matrix. The gel-like matrix is then stabilized by protein and lignin (Pettolino et al. 2012; Ochoa-Villarreal et al. 2012). The unique chemical structure of cell wall complex could be detected by Fourier transform infrared (FTIR) spectroscopy, which is a chemical analytical method capable of detecting minor change in chemical structure (Abidi et al. 2014). Studies have been conducted to associate spectral structural profiles to feed nutritional and digestion profiles (Yu 2005b; Lei et al. 2018; Rahman et al. 2019). However, there is limited study to use FTIR to detect the change of spectral structural feature of feed during the process of rumen degradation and intestinal digestion. Similarly, change of protein related spectral feature during rumen degradation and intestinal digestion is also with little attention. According to the researches (Duodu et al. 2003; Yu et al. 2004b; Yu 2005b), protein secondary structure is associate with digestibility of feed. As a result, change of secondary structure and other protein related spectral feature may also associated with digestion and nutritional features of feed.

2. LITERATURE REVIEW

2.1. Investigation significance

In recent decades, the production of pulse crops in Canada has increased at an astounding rate (Bekkering 2011), and it is especially true in western Canada (Phelps 2017). Faba beans as a specie of pulses also seen great increase in its production due to the improvement of technology and the expansion of market. As a result, using faba beans to substitute some feeds ingredients shows its economic values. Although faba bean exhibits its potential to be used as an alternative feeds resource. However, little information has been obtained about chemical and nutrient profiles, specific feed characteristics, available N to energy synchronization, and true nutrient utilization and availability, especially for the currently developed varieties/lines of faba beans grown in western Canada. Furthermore, according to the research of Caballero (1989), in the beginning of pod-filling stage leaf and stem contribute to 75% of total biomass while the contribution of faba bean seed to the total biomass of faba bean forage rose from 8% to 59% with the advance of the pod-filling stage, and the content of crude protein in seed and tannin in leaf also dramatically changed during the period. Therefore, studying different partitions of faba bean separately can inform us detailed nutritional characteristics of faba bean to ruminant and help us to determine the best stage to harvest the forage.

The process of rumen degradation is vital in determining the amount of nutrient could be utilized by animal. Empirical model established by wet chemistry analyses is mainly used for predicting feed nutritional value. However, feed spectral profiles are also changed during the process of digestion (Xin and Yu 2013b, 2013c, 2014) and could be used to evaluate feed quality and digestion process.

2.2. History of faba bean production

Faba beans *(Vicia faba* L.) are also known as horse bean, broad bean, windsor bean. It belongs to the oldest crops in the world and it has been planted around the world for thousands of years (Singh et al. 2013). Major differences can be found between *V. faba* and other species belonging to the *Narbonensis* complex *(V. narbonensis, V. galilea, V. johannis,* and *V. hyaeniscyamus)* (Zohary and Hopf 1973). Although where the faba beans originally from is still under debate, it is generally agreed that *Vicia faba* L. was geographically originated from Near East and the subspecies *V. faba paucijuga* is its original form (Duc 1997). 5000 years ago, faba bean was used as a food resource in China and 2000 years later, it was cultivated by Egyptians, after that it was imported to Greeks and Romans (Singh et al. 2013). Nowadays, faba bean has been dispersed globally and mainly used as a food resource in industrialized countries including China, Ethiopia and Egypt and China is the country with the highest production at 2×10^6 ha per year (Duc 1997). But in the United States, Canada and northern Europe, faba bean is planted mainly as a feed resource (Duc 1997; Singh et al. 2013). In 2006, the global planting area of faba bean reached 2.6 million ha and most of the producing area was in China, followed by Ethiopia and Europe (STAT 2009; Jensen et al. 2010).

In Canada, the first commercial production of faba bean was in 1972; after that year although the yield fluctuated, the general trend was upward. Before 21st century, the content of anti-nutritional factors in faba bean is the main reason of relatively low production of faba bean. In 2002, the progress made in plant breeding leaded to the presence of tannin-free genotypes, making faba bean more acceptable for both food and feed industry. After that, faba bean was widely planted in western Canada especially in provinces of Alberta, Saskatchewan and Manitoba (Duc et al. 1999; Oomah et al. 2011). In 2012 the cultivation area in western Canada provinces was less than 20 thousand acre, however, in 2015 the cultivation area in Saskatchewan and Alberta soared to more

than 70 thousand acre (Phelps 2017). In western Canada, faba bean is mainly planted to utilize its seeds in local feed market (mainly for monogastrics) and in global food market. Faba bean usually acquire high revenue when export to global market, however, faba bean seed with large seed size and good quality are required for exporting but in Canada faba bean is often of lower quality because of climate limitation (Robinson 2018). As a result, export market is not stable in Canada. In addition, in food market high tannin faba bean with large seed size is utilized while in feed market low tannin faba bean is utilized to avoid the adverse effect of tannin. Therefore, farmer in western Canada should clarity their target market and aware of potential risks.

In Saskatchewan two types of faba beans are mainly cultivated and they are distinguished by the content of tannin. The tannin varieties of Taboar, CDC Fatime, Malik (FB9-4), CDC SSNS-1, Florent, Fabelle (low vicine) and Vertigo with brown seed coat and black dot have 8% to 9% of tannin. The low tannin (zero) varieties are Snowdrop, Snowbird, Imposa, and Tabasco with 1% of tannin and they have white flower and cream seed coat (Rodriguez Espinosa 2018).

2.3. General information about faba bean

2.3.1. Plant and seed characteristics

The *Vicia faba* grows straightly with thick and erected stem to hold the height of the plant up to 1 to 1.5 m and the robust tap root branches with plenteous secondary roots spreading 0.6 m underground; the leaves of faba bean are made up with two to six not twining or slightly curled leaflets; faba bean flowers are prolific with white purple or pink colors and only one to six pods are produced on clusters (Hanelt and Mettin 1989). Pods of faba bean contain two to eight seeds and are up to 10 centimeters long, 1 to 2 centimeters wide. When mature, the color of pods and seeds getting darker. Seeds weight per thousand seeds usually ranges from 400 to 800 grams, and

food market usually demands seeds with size more than 650 grams per thousand seeds (Saskatchewan Pulse Growers 2020).

2.3.2. Growing characteristics

Faba bean is an annual legume and the optimal planting environment is well-drained loam or clay soils with optimal pH between 6.5 and 8.0 (Jensen et al. 2010; Phelps 2017). Moreover, faba bean is more resistant to acid soil type than most legumes (Hekneby et al. 2006; Idris 2008; Singh et al. 2013). Soil salinity stress is a global issue limiting the productivity of crop. In terms of the effect of soil salinity to the production of faba bean, it is found that high concentration of both Na^+ and Cl^- reduce the growth of faba bean and faba bean is more sensitive to the high concentration of Cl^- (Tavakkoli et al. 2010). In addition, faba bean has high requirement of P because of strong ATP demand and high potassium supply in the soil can alleviate the adverse effects of water shortage and have positive effect to N fixation (Sangakkara et al. 1996).

Normally, faba bean flowers at 45 to 60 days after planting and the harvest period reaches around 70 days after flowering (Phelps 2017). Before seedlings is fully accomplished, faba bean is sensitive to water supply, and it takes more than 20 days to emerge (Oplinger et al. 1989). The main pollinators of faba bean are bumblebees and around 35 % of faba bean are cross-fertilization (Stoddard and Bond 1987).

The *Vicia faba* can tolerate cold weather up to -15 ℃, but during its flowering, it is sensitive to heat and dry and the ideal annual precipitation for faba bean cultivation is around 650 to 1000 mm (Phelps 2017).

2.3.3. Nutritional features

Faba bean has seeds rich in protein ranging from 247 to 372 g/kg DM in genetic resources and the protein contents trait is highly heritable (Phelps 2017). Starch is another main nutrient in faba bean

seeds; however, it has negative correlation with protein content, with mean content of 423 g/kg DM (Duc et al. 1999). Faba bean is also a good source of mineral, and the mineral contents was found to be higher in low-tannin faba bean, which are highly heritable, in addition, faba bean had 2-3 fold higher Ca than chickpea and lentil, 14 to 30% higher Mg than pea and lentil, 9 to 18% higher K than pea and lentil and 34% higher Cu than pea but the concentration of Se was lower than other legume species in western Canada (Khazaei and Vandenberg 2020). Besides being used as food and feed resource, faba bean also shows its advantage in fixing atmospheric nitrogen by symbiosis with Rhizobium bacteria under broad climate conditions and crop rotations (Köpke and Nemecek 2010). Faba bean benefits subsequent crops by maintaining N pool in the soil with high level. Although the high N-fixing ability, faba bean should not be planted in the same area more frequent than every fourth year or be planted after consequent year of other pulses to avoid root and stem rot diseases. In addition, faba bean is also vulnerable to pests (Jensen et al. 2010).

With different single seed weight, two types of varieties are distinguished. V. faba major or broad beans are cultivars with large flatten seeds weighting from 1 to 2 g DM per seed while V. faba minor or field beans or horse beans are cultivars with smaller and round seed weighing from 0.4 to 0.8 g DM per seed (Crépon et al. 2010). However, faba bean contains antinutritional factors concerning both human and animal nutrition. As for animal nutrition, the seed of faba bean contains vicine and convicince that have been demonstrated to have anti-nutritional effects on the growth of monogastric animals in several studies (Grosjean et al. 2001; Crépon et al. 2010). As for human health, faba bean seeds have been found to contain divicine and isouramil that are deleterious to people who carry common genetic defect, to specify, faba bean can cause Favism which is an acute hemolysis in G6PD-deficient human individuals (Crépon et al. 2010).

2.3.4. Faba bean as human food

Faba bean has been extensively used as food resource in mid east, Africa, and part of Asia, especially in China, which contribute to the majority of the world faba bean consumption. Seeds of faba bean, because of its high-quality protein and starch sources and various vitamins and minerals compositions are mainly used as green vegetable in these countries. In sub-tropical and temperate regions, faba bean ranks the fourth important legume after dry beans, dry peas and chickpeas (Alghamdi 2009). For food market, faba bean cultivars (broad bean) with larger seed size was mainly consumed. The global broad bean production was around 5 million tons and China production accounts almost half of it. The major reason for the hugest consumption of faba bean in China is partly because of the popular of Sichuan cuisine around the country; fermented faba bean is the most important constituent of a spicy source-Douban Jiang that commonly used in Sichuan cuisine. As a result, faba bean is of huge demand in China. The second largest country of faba bean production is Ethiopia, and around 600,000 tons of faba bean is produced every year (Government of Saskatchewan 2020) Globally, the largest faba bean importer was Egypt, with around 500,000 of import every year; and the largest exporter were Australia, France and UK.

2.3.5. Main antinutritional compounds in faba bean

Although faba bean is a great source of high quality carbohydrate, protein, minerals and vitamins, its nutritional values can be offset by the antinutritional factors such as phytic acid, α-galactosides, trypsin inhibitor, lectin, pyrimidine glucosides (vicine and convicine) and condense tannin (under certain circumstances). Efforts have been made to improve faba bean nutritional quality by eliminating antinutritional factors of faba bean with breeding (Helsper et al. 1993; Khazaei et al. 2019).

Tannins are plant secondary metabolizes widely distributes in various plants protecting plant from pesticide predation and involving in plant growth regulation. Tannin is located in seed hulls of

faba bean. Normally, tannin needs to be hydroxylated and polymerized (with molecular weight more than 500) to be capable of sufficiently binding with protein and other polymers to become stable complex under desired chemical environment (Sinha and Kumar 2018). According to its hydrolytic ability, two classes of tannins, hydrolysable tannins and condense tannins are identified (Rodríguez-Espinosa et al. 2019). Hydrolysable tannin is readily degradable, and its degradation product is gallic acid which does not influent animal performance. Condense tannins are polymers of flavonoids, and the adverse effects of condense tannins to animal are because its astringency affects feed intake and its binding ability with protein affects protein digestion and absorption thus decreasing feed values (Sinha and Kumar 2018). However, according to the research of He et al.(2019), for ruminant diet with high protein degradation rate, less than 5% of total DM intake of condense tannins is able to avoid extra degradation of protein in the rumen and waste of high quality feed protein as ammonia. As a result, tannin is advantageous rather than harmful for ruminant.

Phytic acid is a six-fold dihydrogen-phosphate ester of inositol. Phytic acid interacts with protein to prevent protein from effective digestion and it is also found to lower mineral availability in non-ruminant but in ruminant, microorganism in the rumen is able to hydrolyze it (Sharma and Sehgal 1992).

α-galactosides are soluble, non-reducing sugars widely distributed in plant storage organ. It is the second most abundant soluble sugars in plant being considered as sucrose derivatives (Martínez-Villaluenga et al. 2008). The main adverse effect of α-galactosides in faba bean is because of its high proportion of hindgut fermentation to cause flatulence in monogastics (Martínez-Villaluenga et al. 2008).

Vicine and convicine (VC) are pyrimidine glycosides presenting in the cotyledons of faba bean; their metabolites in the small intestine divicine and isouramil can arise favism for people who has genetic deficiency in their red bloods cells (Gutierrez et al. 2006). In animal nutrition, VC are found to decrease apparent metabolizable value of faba bean in broiler chicken and affect egg quality and egg production rate in laying hens (Gutierrez et al. 2006), and diminishing feed efficiency in pig (Khazaei et al. 2019).

Trypsin inhibitors from faba bean seeds induce release of cholecystokinin in the gut leading to a major loss of S-containing amino acids, which in turn causes pancreatic hypertrophy and depression of growth (Savelkoul et al. 1992). Lectins are glycoproteins binding with specific proteins and sugars to cause agglutination of red blood cells (Savelkoul et al. 1992).

2.4. Faba bean use as animal feed

2.4.1. Use in monogastrics

Alternate legume sources have been extensively tested for possibility of substituting soybean meal (the most commonly used protein sources for pigs) in order to acquire more flexible and profitable feeding management. Faba bean seeds which are good source of protein, are also under consideration. The anti-nutritional content of condense tannin is the major concern for faba bean inclusion in the diet, and according to the research, the condense tannin of faba bean in pigs diet have an adverse effect to protein utilization by increasing endogenous protein excretion and decreasing dietary protein digestion (Jansman et al. 1995). For faba bean with low or zero tannin contents, it is found that inclusion of zero tannin faba bean up to 30% did not adversely affect average daily gain or average daily feed intake but a reduced feed efficiency in the grower phase and reduced lean thickness were observed (Zijlstra et al. 2008). Similarly, inclusion of faba bean and pea for up to 30% in finisher and grower diets does not affect carcass quality and overall

performance, although slightly reduce of growth rate in finisher pigs (Smith et al. 2013). Although the possibility of including faba bean in pig diets is proved, the digestibility of major limiting amino acid is still lower than that of soybean meal, but it can be improved by pre-treatment of extrusion and dehulling (van der Poel et al. 1992; Mariscal-Landín et al. 2002). Overall, the prevalence of low-tannin genotype of faba bean production today gives promising future for faba bean to be used in monogastrics diet.

2.4.2. Use in poultry

When legumes seeds are applied to poultry feed, antinutritional factors are always the major concern. The negative effects of condense tannin and vicine and convicine (VC) on energy utilization were additive and only condense tannin affects total tract protein digestive (Vilariño et al. 2009). In addition, VC in faba bean seeds will lead to reduced egg sizes and total production when fed to laying hen. As a result, when apply in poultry diet, faba bean cultivars with low tannin and VC contents should be considered. Fortunately, the progress made in breeding provides us with choices of faba bean with low tannin and VC contents, which makes application of faba bean in poultry feed possible. In the study of Perez-Maldonado et al. (1999), when faba bean was fed to layer, egg production was lower for faba bean based diet compared with field peas, chick peas and sweet lupins based diets. Consequent experiment about the optimum inclusion level of legumes for broiler found that faba bean based diet acquires better growth rate and feed efficiency than sweet lupins and chick peas and optimal inclusion rate for broilers was recommended for 200g/kg, moreover steam pelleting treatment of the diets gave better results (Farrell et al. 1999). Another study was conducted to feed faba bean to broilers in both mashed and pelleted forms and found that pelleted faba bean did not impair growth rate and feed efficiency but growth rate and feed efficiency decreased linearly with increasing mashed faba bean (Gous 2011). Application of faba

bean to partly replace soybean in organic chicken was also tested, and the results show that it is inapplicable to use faba bean in organic chicken as nutritional requirements can not be met (Bosco et al. 2013). Crépon et al. (2010) suggest that use of faba bean cannot exceed 7% of the diet with high VC content, but when VC content is low, the inclusion rate can be up to 20%. Overall, application of faba bean in poultry diet is application when cultivars with low VC and tannin contents are used and faba bean is pelleted.

2.4.3. Use in ruminants (beef and dairy cattle)

Because of the function of rumen microbiota, condense tannin content in faba bean is not a concern for ruminant, instead it slows down protein digestion and increases protein utilization when condense tannin level is lower than 5% of total DM intake (He et al. 2019). Most of the studies have been concentrated on feeding faba bean seeds to ruminants, only limited studies were conducted to test the feeding value of faba bean forage and faba bean hull. Use of faba bean seeds to substitute soybean meal in ruminants' diet have acquired good results. It is also found that by using faba bean seeds to replace soybean meal, the feed consumption, animal growth and animal products production of ruminants can be maintained (Crépon et al. 2010; Cherif et al. 2018; Mendowski et al. 2019). Use of faba bean hulls in the diet is not suggested because of cellulose content is high (50% DM) and most of protein is rapidly degradable (Minakowski et al. 1996). The nutritional value of faba bean forage is also tested, and limited studies were conducted to compare faba bean silage with commonly used corn and alfalfa silage. The study of Mustafa and Seguin (2003) tested the ensiling ability of faba bean and according to the results, faba silage compared with soybean silage and pea silage had the highest CP content, similar ruminal NDF digestibility and lower CP and DM ruminal digestibility. In addition, McKnight and MacLeod (1977) compared feeding value of faba bean and grass-legume silages, and similar milk protein, solids contents and

milk production was observed, which indicates that whole plant faba bean may be a satisfactory alternative forage.

2.4.4. Faba bean seeds as animal feed

Most of the application of faba bean in animal science feed have been focused on the use of faba bean seeds as a protein resource. Compared with other legume raw seeds, the solubility and degradability of nitrogen fraction are higher in faba bean (Sauvant et al 2004). However, the antinutritional factors in the seeds are major concern. For animal feed, small seeds cultivars with low tannin contents are mainly used which are cultivars of Snowdrop, Snowbird, Imposa, and Tabasco.

2.4.5. Faba bean whole plant as a forage source

Forages are important feed resources for ruminant. They provide not only energy and protein but also fibers that are crucial in maintaining rumen health. The most commonly used forages in ruminant diet are legumes, corn silage and grasses (Linn and Kuehn 1996). Forages are provided to animals in different forms, which include dry forage (hay), silage, pasture and green-chop. Hay is the traditional forage during the barn feeding season and is made to be easily transported and safely stored. However, hay producing is influenced by weather conditions and requires more difficult mechanization compared with silage which entails less weather hazard. Pasture, on the other hand, varies in feed quality that depends on plant species, growth stage and available material. Forages are difficult and expensive to transport because of bulkiness, as a result, alternatives are needed when common used forages are in short (Farminfo 2002). Faba bean has been shown its potential to be a good alternative for forage resource (Sheaffer et al. 2001). Faba bean crop is a good source of protein and fiber. Furthermore, faba bean is resistant to different weather condition and faba bean forage has been reported to produce 7.8 tons ha^{-1} DM yields with CP concentrations

of 18% (Fraser et al. 2001; Singh et al. 2013). However, focuses have concentrated on faba been seed as an optional protein and energy feed resource in the past. Limited attention has been paid to the use of faba bean whole plant as a forage resource.

According to Fraser et al. (2001), different growth stages influence yield and ensilage potential of faba bean silage and the optimum growth for faba bean silage occurred at 14 weeks of growth. Researches have been conducted to ensilage whole plant faba bean, according to the results, both expected lactic acid fermentations and unsatisfactory clostridial can happen during fermentation, hence, additives (or co-ensilage) and proper wilting is needed to maintain a lactic acid dominant fermentation (Pursiainen and Tuori 2008; Borreani et al. 2009).

Research to compare rumen degradation kinetics of faba bean with soybean whole plant silage was also conducted, and the results show different chemical composition of these legume silages leads to variation in DM and CP degradability (Mustafa and Seguim, 2003).

Faba bean silage was also compared with grass-legume silage in terms of chemical compositions. According to McKnight and MacLeod (1977), faba bean was higher in protein (20.1 vs. 16.l7 %), lower in crude fiber (25.0 vs.29.6 %), ether extract (1.8 vs.3.2 %), ash (7.1 vs. 8.0 %), calcium (0.32 vs. 0.85 %) and phosphorus (0.39 vs. 0.43 %) compared with grass-legume silage. In addition, when faba bean and grass-legume silage were feed to cows, similar milk protein, solids contents and milk production were observed, which indicates that whole plant faba bean may be a satisfactory alternative forage.

2.5. Function of rumen microbiomes to the digestion of feed

2.5.1. Function of ruminal bacteria

The biggest advantage of ruminants over monogastric is their ability to utilize plant cell wall, and this advantage enables us to feed growing human population by acquiring animal protein from

resource that human cannot utilize. Rumen microbiomes which include bacterial, protozoa, anaerobic fungi, ruminal archaea and bacteriophage are the key to utilize plant cell wall. The successful culture of some anaerobic bacterial species by the father of rumen microbiology Robert Hungate in 1960s enabled us for the first time to take a glance of the mysterious world of rumen microbiology (Huws et al. 2018). After that, rumen microbiomes began to attach great attention by the scientists. Within the vast population of rumen microbiomes, ruminal bacteria are the most abundant and diverse group. Rumen bacteria are involved in enzymatic digestion of starch, fibers, proteins and lipids. Some of the ruminal bacteria have substrates specificity, which have strict restriction to the substrates that can be used, while some of the ruminal bacteria can accept various substrates. For example, only starch and its degradation products are utilized by *Ruminobacter amylophilus*, while *Butyrivibrio fibrisolvens* have a broad range of preys from cellulose to pectin (Hobson and Stewart 2012).

As for the degradation of cellulose and hemicellulose, the cooperation of three glycosyl hydrolases (GH) produced by cellulolytic microorganisms are required, which include endoglucanases, exoglucanases and β-glucosidases (Huws et al. 2018). Specifically, according to the proposed way, endoglucanases attached on amorphous regions of cellulose to create sites for exoglucanases attachment on crystalline region of cellulose (Lynd et al. 2002). The consequent outputs of cellobiose and short-chain cellodextrins are hydrolyzed by β-glucosidases to yield glucose (Huws et al. 2018). Within the 14 GH families that have endoglucanase function, rumen microorganisms are mainly producing family 5 and 9 while exocellulases of rumen origin are family 6, which are only found from anaerobic fungi (Huws et al. 2018). As for xylanases, endoxylanases of rumen origin, they are mainly from family 10 and family 11, and β-xylosidase are from family 43. The well-studied ruminal bacteria responsible for production for cellulodases and hemicelludases are

Fibrobacter, Ruminococcus, Butyrivibrio, Prevotella, and they function together with anaerobic fungi in a form of cellulosome.

Fermentation of starch is the rumen involves cleave of α-1,4 and α-1,6 linkage of amylose and amylopectin with α-amylase produced by rumen microorganisms, especially amylolytic bacteria. Anaerobic fungi and protozoa are also involved in digestion of starch but found not essential. However, engulf of starch granules by protozoa, slows down the fermentation rate, thus reducing the risks of acidosis (Hobson and Stewart 2012). The bacteria involved in starch digestion are *Ruminobacter amylophilus, Prevotella ruminicola, Streptococcus bovis, Succinimonas amylolytica,* some strains of *Selenomonas ruminantium, Butyrivibrio fibrisolvens, Eubacterium ruminantium* and *Clostridium spp* (Hobson and Stewart 2012).

Ruminal bacteria also involve in the biohydrogenation of lipid in the rumen.

2.5.2. Function of ruminal protozoa

Ruminal protozoa in the rumen includes ciliates protozoa and flagellate protozoa depending on their morphological characteristics. Compared with ciliate protozoa, flagellate protozoa is smaller in size (3-12 μM versus 10 to 200 μM), and fewer in number, thus contributing minimal to overall ruminal fermentation (Owens and Basalan 2016). Being the predators in the rumen, ciliate protozoa possesses the largest biomass of total ruminal microorganisms. Although ciliate protozoa function in enzymatic digestion of almost all substances in the rumen, difficulties in culturing protozoa *in vivo* is main obstacle for fully understanding its function in rumen fermentation. The research about function of protozoa is mainly depending on defaunation in different feeding conditions and the results are under debate. Generally, when high protein diet is fed, protein efficiency is increased as predation of protozoa is removed; when high fibrous diet is fed, fiber utilization is undermined as lack of enzymatic digestion of cellulose by protozoa. However, when

high starch diet is fed, remove of protozoa may lead to increasing risk of acidosis because of rapid fermentation of readily degradable carbohydrate contents.

2.5.3. Function of ruminal anaerobic fungi

Anaerobic fungi are important to the digestion of carbohydrate in the rumen by both enzymatic digestion and physical penetration of rhizoids during the development of thalli. Anaerobic fungi also involve in digestion of protein by producing proteases, however, the production of proteases varying significantly is anaerobic fungal isolates (Gruninger et al. 2014). Anaerobic fungi are able to utilize plenteous sources of carbohydrate in the rumen expressing at the production of cellulases, hemicellulases, pectin lyases, amylases and even phenolic esterases. Anaerobic fungi show their great importance in ruminal fermentation by facilitating other rumen microorganisms. The break down of linkage between hemicellulose and lignin by esterases produced by anaerobic fungi enables better digestion of hemicellulose. The physical penetration of plant cell wall by rhizoids creating sites for bacterial attachment. The end-products of fungal digestion formate and H_2 are also precursors for methane thus benefits methanogens in the rumen.

2.5.4. Function of ruminal archaea

Most ruminal archaea are methanogens, which utilizing fermentation products of mostly CO_2, H_2 and partly formic acid and methylamines as substrates to produce a potent greenhouse gas-methane. The warming global climate caused by emission of greenhouse gases have arouse great attention by people. As ruminal methane production accounted for about 17% of total global methane production (Knapp et al. 2014), ruminal archaea have been attached great attention in last decade. Compared with other ruminal bacteria, archaea diversity is very small, only according for 6.8% of total ruminal SSU rRNA and less than 3.3% of total rRNA and only 0.7% of total archaeal

gene.(Patra et al. 2017). The archaea in the rumen seems to be not diverse; among the cultured isolates, most of them are from family *Methanobacteriaceae*.

Both free-living methanogens and methanogens endosymbiotic within ciliates are found in the rumen. The majority of the methanogens are live freely within the biofilm utilizing H_2 produced by bacteria and are protected from anti-methanogenic inhibitors (Patra et al. 2017). Besides symbiotic within the ciliates, the H_2 released from ciliates also attach archaea residue outside of the membrane. Same as ciliates, anaerobic fungi also possess a unique organelle called hydrogenosomes, which producing H_2 by malate oxidization; however, it is still hard to make the conclusion that there are archaea endosymbiotic within fungi (Patra et al. 2017).

2.5.5. Function of ruminal bacteriophage

Relative limited information is available for bacteriophage in the rumen because of little successful cultivation of bacteria *in vivo* and paucity of available sequences of virus. The functions of bacteriophage to rumen function are influencing bacteria population and diversity and acting as media for horizontal gene transfer (Gilbert and Klieve 2015). The effects of bacteriophage to animal performance can be both advantageous and disadvantageous and requires more researches to fill the gap of knowledge about bacteriophage.

2.6. Feed evaluation methods

2.6.1. Chemical evaluation of feeds

The analysis of feedstuff is to acquire feed chemical compositions for formulating animal diet that can have predictable animal productions from livestock (Van Soest and Robertson 1979). After the establishment of the feed proximate analysis in 1860s by German scientists Hunneberg and Stohman, the feed proximate analysis has been under continuous revisions and updates and its basic concepts have been utilized in feed analysis until today. Instead of acquiring the content of

a single chemical component, feed proximate analysis gives us the contents of groups of chemical components with similar chemical structure and conformation that have specific biological function to animals. According to feed proximate analysis, feed compositions are divided into water, crude protein, crude fat (ether extract), crude fiber, nitrogen-free extract, and crude ash. However, this approximate fractioning of feedstuff composition cannot fulfill the needs of knowing more precise compositions of feed, especially in its partitioning of feed fibrous fractions. According to Van Soest and Robertson (1979), the crude fiber method is of concern for not only ruminant but also monogastrics and human nutrition and it needs replacement with more scientific and accurate method. Specifically, the contents of cellulose, hemicellulose and lignin that make up the cell wall are actually higher than crude fiber content because from crude fiber method, considerable contents of cellulose, hemicellulose and lignin are dissolved. Consequently, the determination of nitrogen-free extract can be problematic. As a result, the detergent system, which further divide neutral detergent fiber (NDF) into acid detergent fiber (ADF) and acid detergent solubles (ADS) was applied. Moreover, the content of water-soluble carbohydrate and starch are determined today. In addition, the accuracy of the parameter 6.25 used to convert feed nitrogen content into crude protein is affected by the proportion of non-protein nitrogen content in the feed. Hence, the content of non-protein nitrogen in feed is commonly determined especially for ruminants.

2.6.2. Application of Cornell Net Carbohydrate and Protein System (CNCPS 6.5) for feed evaluation

Cornell Net Carbohydrate and Protein System is a mathematical model that predicts animal requirement based on physiological status, production purpose and environmental effects and it evaluates feed nutrients supply with not only chemical composition but also rumen microbiology

and degradation kinetics (Fox et al. 2004). The first edition of Cornell Net Carbohydrate and Protein System (CNCPS) was come out as a series of four papers in 1992 and 1993 (Russell et al. 1992; Sniffen et al. 1992; O'Connor et al. 1993; Fox et al. 2004). After that, CNCPS has gone through continuous updates and nowadays the commonly used version is CNCPS 6.5 or 6.55 for both practical production and scientific research.

Before the release of CNCPS sub-model, use of the proximate analysis method to evaluate feed quality fails to associate complicated rumen microorganism compositions and competition of nutrients between passage and digestion rates. During that time the NRC system had some limitations in predicting microbial protein production: (1) considered microbial protein production driven by total digestible nutrient (TDN) instead of available carbohydrate; (2) assumed a constant growth efficiency for microorganism; (3) did not consider the effect of passage rate to actual feed digestion; (4) did not partition rumen microbial population according to fermentation characteristics and (5) did not integrate nitrogen digestion to energy availability (Russell et al. 1992). When the first paper of CNCPS model was released in 1992 (Russell et al. 1992), it put complicated microbial digestion into consideration and proposed a kinetic sub-model that divides microbial ecosystem into structural carbohydrate (SC) fermenter and non-structure carbohydrate (NSC) fermenter; hence it overcame the shortage of NRC model and provided a useful model for ruminant production. The second paper of the series raised a sub-model that predict degradation of carbohydrate and protein of feedstuffs and the corresponding partitioning of feedstuff carbohydrate and protein fractions (Sniffen et al. 1992). The third paper of the series predicted the nutrient requirement and feed intake based on different physiological states and production (Fox et al. 1992). The fourth paper of the series predicted the amino acids requirement and absorption

for ruminants (O'Connor et al. 1993). Overall, the release of first four papers set foundation for the CNCPS model.

Since the first release of the CNCPS model it has gone through continuous improvement; the currently used model CNCPS 6.5 or 6.55 version was released in 2015 and feed library and prediction equation have been refined for more precise prediction (Higgs et al. 2015; Van Amburgh et al. 2015).

2.6.3. Energy value estimation in feed ingredients

Energy values of feedstuff are important parameters for evaluating feedstuff quality, especially the quality to promote lactation in dairy cattle and meat production in beef. Unlike other nutrients, it cannot be measured in laboratory, instead, it is predicted by equation derived from regression equation. The prediction equation of National Research Council (NRC) is the one that widely accepted as authoritative.

Previous edition of NRC-1989 system has major drawback in experimentally determining the total digestible nutrient (TDN) value based on feed compositions and assign TDN values to feedstuff with similar composition. However, the assigned TDN value can be inaccurate. In addition, a constant 8 % of deduction are assigned as all cows are believed to consume at three times maintenance (NRC 2001). The digestibility of feed is reducing with increasing intake. In addition, feed energy values should be calculated based on actual feedstuff and its function in diet. As a result, the latest NRC-2001 model overcame the drawbacks of NRC-1989 model by calculating TDN value based on feed compositions and calculating net energy for lactation (NE_L) based on actual intake (NRC 2001). The consequent digestible energy (DE) value is then calculated based on its feed categories and actual intake. The corresponding metabolizable energy and net energy are then calculated from DE value at actual intake.

2.6.4. In situ technique for estimation of rumen degradation kinetics of feed nutrients

Matching energy supply with N release to maximize microbial protein synthesis and feed efficiency has been recognized for a long time. However, before the invention of *in situ* technique to study the degradation characteristics of feed in the rumen, the synchronization of nitrogen and energy in the rumen cannot be fully explored. In 1979, the study of Ørskov and McDonald (1979) proposed an artificial-fiber bag technique to determine the rate of degradation of feed in the rumen using cannulated cattle. Along with the technique is the mathematical model used to evaluate kinetics of feed composition that degraded at different time points in the rumen. After the release of this paper, this technique has been widely used in ruminant research, for example to study the synchronization of nitrogen to energy in the rumen (Sinclair et al. 1993).

2.6.5. Three-step in vitro technique for evaluation of intestinal digestibility in feed nutrients

With the information of microbial protein synthesis and undegradable protein after ruminal digestion, it is necessary to estimate its contribution to animal production through small intestinal digestion. The commonly used *in vivo* method requires fistulated animal thus is labor-intensive and costly and involves animal variance (Stern et al. 1997). In addition, the lack of empirical data about intestinal protein digestibility was recognized (Stern et al. 1997). As a result, to overcome the disadvantages of *in vivo* method and to fill in the gap of protein intestinal digestibility, several methods (bioassays; *in situ* mobile-bag technique and *in vitro* technique) have been utilized in estimation of intestinal protein digestibility; among them three-step *in vitro* method is of commonly use. Three-step *in vitro* method was firstly proposed by Calsamiglia and Stern (1995b) in 1995, and it has gone through revision in 2006 by utilization of batch incubator (Daisy II) to

save the labor and cost (Gargallo et al. 2006). Nowadays, three-step *in vitro* method as well as Daisy II batch incubator are commonly used in intestinal digestibility estimation.

2.6.6. Prediction of truly digestible protein supply to small intestine and feed milk value in dairy cattle

2.6.6.1. DVE/OEB System

One of the most important drive of production ability of ruminants is so called metabolizable protein which consists of undegradable feed protein; rumen synthesized microbial protein and endogenous protein. To evaluate the metabolizable characteristics of the feed several models have been utilized namely NRC model, the Dutch DVE/OEB system and the France PDI system. Among them, the DVE/OEB system and NRC system are commonly used and undergo continuous revision and update. The DVE/OEB system was firstly proposed in 1991 to replace the digestible crude protein system (DCP) to make the most efficient use of nitrogen (Tamminga et al. 1994). According to Theodoridou and Yu (2013b), the protein supply to the small intestine of dairy cows of a feed is estimated based on DVE/OEB system which constitute of two parts, the DVE and the OEB value respectively. The DVE value is constituted of digestible feed protein, microbial protein, and an endogenous protein loss correction while the equation is described as : DVE = $AMCP^{DVE}$ + $ARUP^{DVE}$ – ENDP, where $AMCP^{DVE}$ is the microbial crude protein that is absorbable; $ARUP^{DVE}$ is the ruminally undegradable feed protein and ENDP is used for correcting the endogenous protein lost during the digestion process. The OEB value is described as equation OEB = $MCP_{RDP}{}^{DVE}$- MCP_{FOM}. $MCP_{RDP}{}^{DVE}$ is the potential MCP synthesis based on RDP, and MCP_{FOM} is potential MCP synthesis using energy from anaerobic fermentation. The $MCP_{RDP}{}^{DVE}$ is calculated as equation $MCP_{RDP}{}^{DVE}$ = CP × [1 - (1.11 × RUP (% CP)/100)].

2.6.6.2. NRC-2001 Model

Similar with DVE/OEB system, the metabolizable protein supply in NRC-2001 model has the same sources. However, different with DVE/OEB system, the endogenous protein is contributed to the metabolizable protein. According to NRC-2001 model (NRC 2001), the calculation is given as below. When RDP exceed 1.18 ×TDN-predicted MCP ($MCP_{TDN)}$, MCP_{TDN} (Potential ruminally synthesized microbial CP) will be calculated as MCP_{TDN} (g/kg of DM) =0.13 × TDN (discounted), When RDP is less than 1.18 × TDN-predicted MCP (MCP_{TDN}), then MCP will be calculated as 0.85 of RDP (MCP_{RDP}^{NRC}); truly absorbed MCP ($AMCP^{NRC}$) will be calculated as $AMCP^{NRC}$ (g/kg of DM) = 0.80 × 0.80 × MCP_{TDN}, where true protein and digestibility of ruminally synthesized microbial CP are both defined as 800g/kg in NRC-2001; $ARUP^{NRC}$ (Truly absorbed rumen-undegraded protein in the small intestine) will be calculated as $ARUP^{NRC}$ = RUP^{NRC} × dRUP (intestinal digestibility of rumen undegraded protein); rumen endogenous protein in the small intestine (ECP^{NRC}) will be calculated as ECP (g/kg of DM) = 6.25 × 1.9 × DM; truly absorbed rumen endogenous protein in the small intestine (AECP) value will be calculated as AECP (g/kg of DM) = 0.50 × 0.80 × ECP; total metabolizable protein (MP) will be calculated as MP (g/kg of DM) =$ARUP^{NRC}$ + $AMCP^{NRC}$ + AECP; the degraded protein balance (DPB^{NRC}), which describe the difference between potential microbial protein synthesize based on energy RDP, will be calculated as DPB^{NRC} (g/kg of DM) = RDP^{NRC} - 1.18 ×MCP_{TDN}.

2.7. Mid-infrared (Mid-IR) spectroscopy techniques in feed science

2.7.1. Mid-Infrared spectroscopy and Fourier transform infrared spectroscopy (FITR)

Infrared spectroscopy is the applicable analytical technique emerged from 1940's and nowadays it has become a powerful tool commonly used in identifying organic compounds, characterizing polymers and studying biological molecules (Stuart 2015). In addition, it is a non-destructive and rapid analytical technique which can be applied in samples of different states such as lipids,

powders, solution, films and gases (Stuart 2015). According to wavenumber range, an infrared spectroscopy works in three main regions: the far-infrared region (wave number less than 400 cm^{-1}), the mid-infrared region (wavenumber between 4000 and 400 cm^{-1}) and the near-infrared region (wavenumber between 13000 and 4000 cm^{-1}). Both near-infrared (NIR) and mid-infrared (MIR) are commonly used in food and feed analysis to obtain both quantitatively and qualitative relationship (Li-Chan et al. 2010). Far-infrared instead is mainly used for the investigation of inorganic substances (Prati et al. 2011). While NIR informs on harmonic and overtone absorptions, MIR acquires the spectra based on fundamental molecular vibration, which explains the reason why MIR gives better insight molecular bonds (Nikolic 2011). In other words, spectra recorded from near-infrared (NIR) range are "sensitive to multitude of compounds and molecular interactions" and NIR is superior to MIR in quantitatively predicting nutrient compositions in grains (Hell et al. 2016; Shi and Yu 2017). However, NIR spectra fail to identify more complex and similar structures, which could be achieved with mid-infrared spectroscopy (Hell et al. 2016). In general, MIR is more commonly used to qualitatively identify and verify chemical compound while NIR is more commonly used to quantitatively predict chemical compositions in the sample (van de Voort 1992).

The introduction of Fourier transform infrared spectroscopy (FTIR) spectrometers has significantly improved the utilization of mid infrared spectroscopy. Compared with conventional IR spectroscopy which utilizes individual wavelength, FTIR spectroscopy makes use of complete source spectrum and thus having several advantages over dispersive type instruments, such as higher signal-to-noise ratio, less scan time and superior scan accuracy (van de Voort 1992). The basic idea of FTIR spectroscopy is to obtain an interferogram by interference of two beams and using mathematical method called Fourier transformation to yield the spectrum (Stuart 2015). The

core component of FTIR spectrometer is the interferometer which consists of two perpendicular plane mirrors, one of which can travel perpendicularly to the other, a beamsplitter bisects the plane of two mirrors, a radiation source and a detector (Stuart 2015). The detail of FTIR interferometer is shown in Figure 2.1.

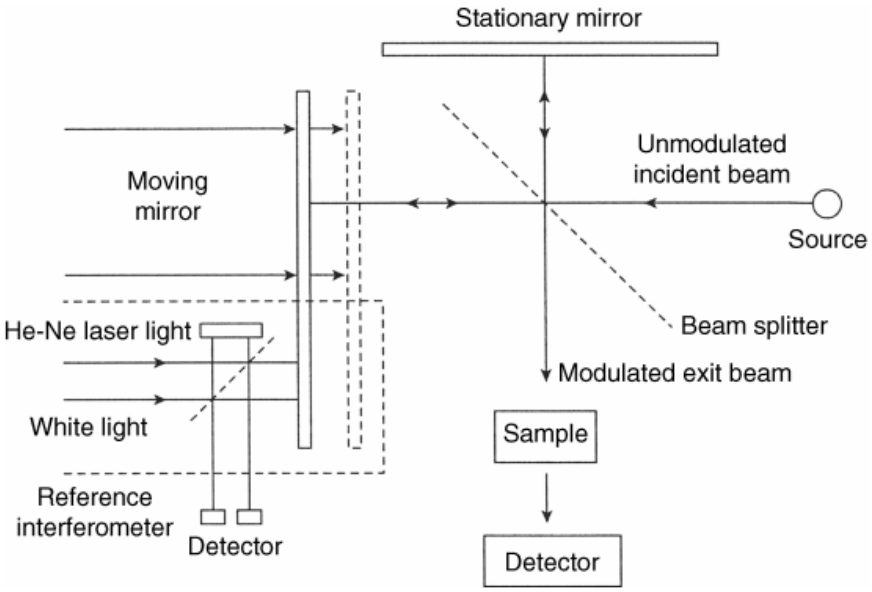


Figure 2.1. Michelson Fourier Transform Infrared Spectrometer (FTIR) interferometer. Source from Stuart (2004).

2.7.2. Basic principles of infrared spectroscopy

The total energy of a molecular consists of translational energy, rotational energy, vibrational energy and electronic energy. When infrared radiation is processed to the organic molecular, the molecular equilibrium stage is broken down, which promotes the rotational and vibrational energy of molecule and lead to the unique absorption of infrared radiation because of the unique chemical bonds and functional groups of organic matter (Colthup 2012). According to Stuart (2015), the characteristics of a group frequency are mainly reflected by its region in spectrum; within the mid-infrared region, it can be further divided into X-H stretching region (ca. 4000 to 2500 cm^{-1}), triple bond region (ca. 2500 to 2000 cm^{-1}), double bond region (ca. 2000 to 1500 cm^{-1}) and the fingerprint

region (ca. 1500 to 600 cm^{-1}) (Stuart 2015). The vibrations in X-H stretching region are generally because of the stretch between the hydrogen atom and atoms like carbohydrate, oxygen and nitrogen, while triple bond stretching absorptions are due to C≡C and C≡N and sometimes X-H stretching, where X is atom with bigger atomic mass like silicon and phosphorus. The vibration in double bond region is generally due to C=C, C=O and C=N stretching with the C=C stretching one of the most noticeable absorption in an infrared spectrum. By associating the unique absorption of IR radiation to its chemical bonds, the chemical composition and structural information can be acquired. Unlike in other regions where each band in the region can be assigned to specific characteristic of a molecule, in fingerprint region, the vibrations are extreme variable and are even unidentical for similar molecules(Stuart 2015). As a result, the spectrum in fingerprint region is unique and can be considered as the fingerprint of the molecule.

2.7.3. Application of MIR technique in feed analysis

Besides the amino acid conformation, proteins are exquisitely assembled to its unique three-dimensional structure (secondary structure) to fulfill its function, and the secondary structure of protein includes mostly α-helix, β-sheet, with small proportion of β-turn and random coils. Since 1950s, the correlation between the secondary structure of protein and the frequency of infrared spectroscopy absorption in so-called amide I and amide II regions has been found; specifically, the vibration in amide I region was found to be related to mainly C=O stretching and partly C-N stretching, while N-H bending and C-N stretching contribute to the vibration in amide II region, in other words, the frequency of amide I and amide II absorptions is related to any hydrogen bonds involved with amide C=O and N-H groups (Jackson and Mantsch 1995). As different proteins have unique secondary structure which can be reflected in the distinctive hydrogen bonding pattern

between C=O and N-H groups, thus the infrared absorptions in amide I and amide II region can be utilized to determine distinguished secondary structure of protein (Jackson and Mantsch 1995).

In feed industry, the feeding value of protein in feedstuff is mainly considered from the perspective of amino acid composition and protein digestibility without taking secondary structure of protein into consideration. However, the feeding value of protein is also influenced by the structure of protein in digestive behavior, nutritive quality and digestibility in animals, as the secondary structure of protein is highly associated with the specific susceptibility to the enzymatic hydrolysis (Yu 2005b, 2007). According to Yu (2004c), feathers are very high in crude protein content, but the protein digestibility is very low; and the low protein digestibility is partly associated with its secondary structure as feather protein have a high β-sheet ratio compared with other grains. In addition, the protein nutritional profiles of feed is found to be closely correlated to its spectral profiles (Li et al. 2016; Lei et al. 2019). As a result, FTIR, which is capable of detecting the secondary structure of feed sample is a valuable tool in assessing the feed value in regard with its protein secondary structure (Yu et al. 2004b; Yu 2005b, 2005c).

Plant cell wall structure is important in determine the nutritional value of feed to ruminant. Different polysaccharides associated with hydrogen bond to form three types of structurally independent polymer network: cellulose microfibrils connecting with non-cellulosic polysaccharides (for example phenolic heteropolymer lignin), gel-like matrix of pectin linkage with other polysaccharides and their cross-linkage with structural protein (Pettolino et al. 2012). Polysaccharides linkage between each other and linkage between polysaccharide and protein and lignin can all detected by FTIR and contributed to unique spectra. In addition, the change of plant cell wall structure by processing or biohydrogenation can also be reflected in its change of

chemical bonds and can be easily detected by FTIR. An example of complicated chemical bonds between lignin and polysaccharides are shown in Figure 2.2.

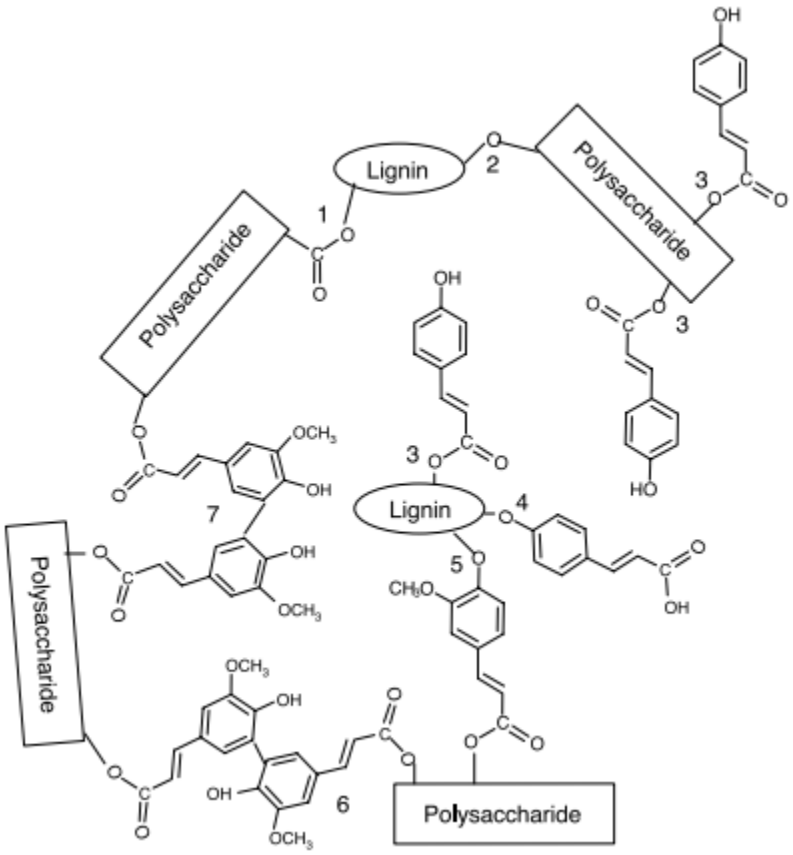


Figure 2.2. A representation of the different kinds of lignin interactions in plant cell walls. A summary of the kinds of aromatic ester and ether cross-links between carbohydrate and lignin. Linkages are: 1 = direct ester linkage, 2 = hydroxycinnamic acid ester, 3 = hydroxycinnamic acid ether, 4 = ferulic acid bridge, 5 = direct ether linkage, 6 = dehydrodiferulic acid diester bridge, 7 = dehydrodiferulic acid diester-ether bridge. Adapted from Krause et al.(2003)

Feed processing is commonly used in feed industry to reduce antinutritional factors, increase feed efficiency and digestibility. The accompanied protein structure alteration during the feed processing was found to affect the digestive behavior, nutrient utilization and availability of protein, but traditional wet chemistry analysis method fails to unveil the internal relationship between the change of protein structure feature and the change in nutritional value. While FTIR is capable of detecting the change in protein without destroy the protein structure, thus FTIR is

proved to be able to give a better insight in understanding the intrinsic protein molecular structural change during processing (Yu 2007; Yu and Nuez-Ortín 2010; Theodoridou and Yu 2013a, 2013c; Yu et al. 2015). In recent years, the by-products of ethanal industry, distillers dried grains with solubles (DDGS) has entered the feed industry as a raw material. Yu and Nuez-Ortín (2010) compared different types of DDGS, such as wheat DDGS, maize DDGS and blend DDGS (wheat: maize = 70:30) regarding the processing related change in IR spectroscopy (α-helix, β-sheet, amide I and amide II and their ratios) and their protein nutritive values (rumen protein digestibility, intestinal protein digestibility and truly absorbable protein in small intestine), results show that the changes of spectral profiles with bioethanol processing were highly associated with its protein nutritive values, and suggest the protein structure features should be considered in evaluating the protein value for the feedstuff.

In addition to detecting the change of protein secondary structure (amide I, amide II, α-helix and β-sheet) of feed during processing and differentiating feed protein quality according to protein spectral profiles, FTIR has also been proved to be able to detect change of molecular structure during gene modification. In recent years, significant progress has been made in gene modification to improve forage quality for sustainable agriculture development and to create tremendous economic benefits (Wang and Ge 2006). Gene modification of forage is mainly focus on manipulating gene expression in important metabolic pathway to acquire better nutritive value, productivity, and improved agronomic characters. However, the change of molecular structure has also been found to happen during gene modification and such changes is related to the nutritive value of forage, and FTIR is capable of detecting such change (Jonker et al. 2012; Lei et al. 2017, 2018b, 2019). According to Lei et al. (2019), the silencing of HB12 and TT8 genes in alfalfa aiming at reducing tannin content in alfalfa, decreased the protein degradation and metabolic

profiles, and the changes in nutritional and digestive characteristic are closely correlated to their spectral features. In addition, Badhan et al. (2014) successfully utilized FTIR to differentiate the alterations in cell wall architecture generated by gene modification.

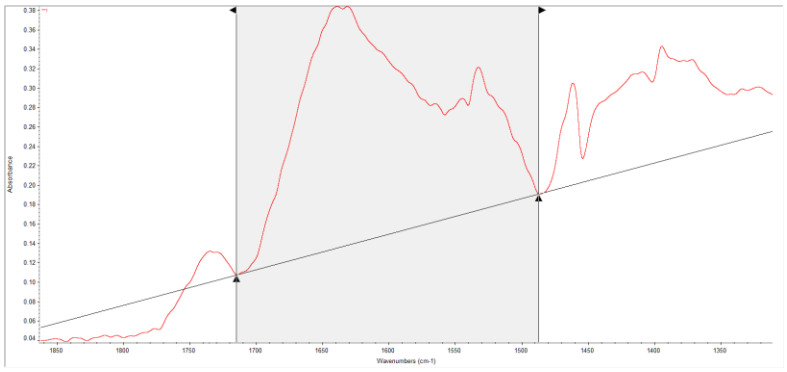
In addition to amide I and amide II regions, other spectral regions have also been found to be highly correlated to nutritional characteristics and structural characteristics of feed. Just like protein, carbohydrate and lipid also have their unique molecular and chemical features, which are in relation to their structural characteristics; specifically, lipid contains C=O carbonyl as well as CH_2 and CH_3 functional groups, while the unique structural chemical-structural features of carbohydrates are OH and CO bonds (Yu et al. 2004b). According to published papers (Himmelsbach et al. 1998; Wetzel et al. 1998, 2003; Yu 2012), the spectral regions related to carbohydrate and lipid have been identified. As for carbohydrate related spectral profiles, spectral region between ca. 1485 and 1188 cm^{-1} is mainly associated with hemi-cellulosic and cellulosic compounds; spectral region between ca. 1292 and 1198 cm^{-1} is mainly associated with cellulosic compounds; spectral region between ca. 1187 and 950 cm^{-1} is associated with total carbohydrate (Yu 2012). As for lipid related spectral profiles, there are four peaks at ca. 2955, 2873, 2922 and 2843 cm^{-1}, which are attributed to CH_2 and CH_3 asymmetric and symmetric stretching vibrations, and lipid carbonyl C=O ester is associated with spectral region between ca. 1774 and 1771 cm^{-1} (Morent et al. 2008; Abeysekara et al. 2012). Unlike in fingerprint region where vibration induced by infrared radiation is unique and can be considered as the fingerprint of the molecule, in carbohydrate and lipid related spectral regions, each band in the region is associated with specific characteristics of a molecule, in other words, the spectral profiles in carbohydrate and lipid related regions reflect more information in intermolecular reaction rather than molecular structure. According to published papers (Abeysekara et al. 2012; Xin et al. 2013, 2014; Xin and Yu 2014),

both the carbohydrate and lipid related spectral features are correlated to nutrient values to some extent, and may be used to predict nutritional supply to animal. In addition, carbohydrate and lipid related spectral features are correlated to nutrient values and may be used to predict nutritional supply to animal (Xin et al. 2013). As a result, FTIR is utilized to reveal the association between internal molecular structure and nutritional and digestive characteristics of feeds, as well as the possible alteration of structure during processing (Rodríguez-Espinosa et al. 2019). However, researches fail to interpret more information in the spectra, thus cannot applying the principle to a broader scale. To be specific, the correlation and regression relationships between spectral feature and chemical and digestive behavior of feed acquired from one treatment cannot be applied to another one, which inhibits its application in the industry as a routine evaluation.

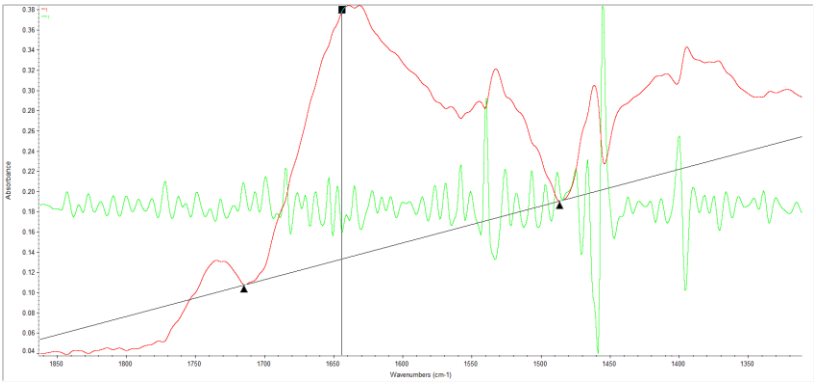
Although NIRS has been approved by the Association of Official Analytical Chemists (AOAC) in quantitatively determination of crude protein, moisture and acid detergent fiber content in feed (Undersander 2006), it fails to detect more subtle difference between molecules. The technical improvement made in Fourier transform instruments and sample preparation in recently years enables MIRS to successfully detect more complex molecules in feed (Sherazi et al. 2007; Allison et al. 2009b, 2009a; Nikolic 2011). According to the study by Sherazi et al. (2007), FTIR successfully distinguished free fatty acids from other lipids and was proved to be an efficient and repeatable method to determine free fatty acids content in poultry feed lipid extracts. In addition, Allison et al. (2009b, 2009a), used FTIR with partial least square regression to predict lignin and hydroxycinnamic acids, nitrogen and alkali index in grasses. As a result, FTIR shows its possibility to be utilized as a quick, non-destructive, and environment-friendly method in quantitatively predict feed composition of some molecules with similar structure which cannot be predicted with NIR and chemical analysis.

2.7.4. Statistical methods for spectra analysis

The analytical methods for spectral analysis utilized in feed analysis are mainly univariate analysis and multivariate analysis. As for univariate analysis, it is straightforward measuring of frequency and intensity (peak height and peak area) that contain feed structural (amide I, amide II, α-helix and β-sheet) or chemical (spectral regions related to cellulosic compounds, structural carbohydrate compounds, lignin et al.) information under the assistance of spectral software like OMNIC or OPUS. In amide region, peaks are always overlap to each other, thus needing the mathematical pretreatment such as Fourier self-deconvolution and secondary derivative. Figure 2.3 gives an example of how the peak height and peak area data is acquired.



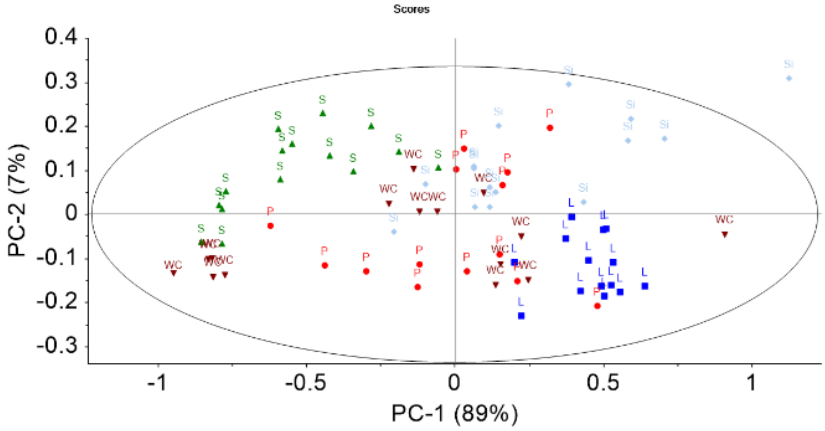
(a)



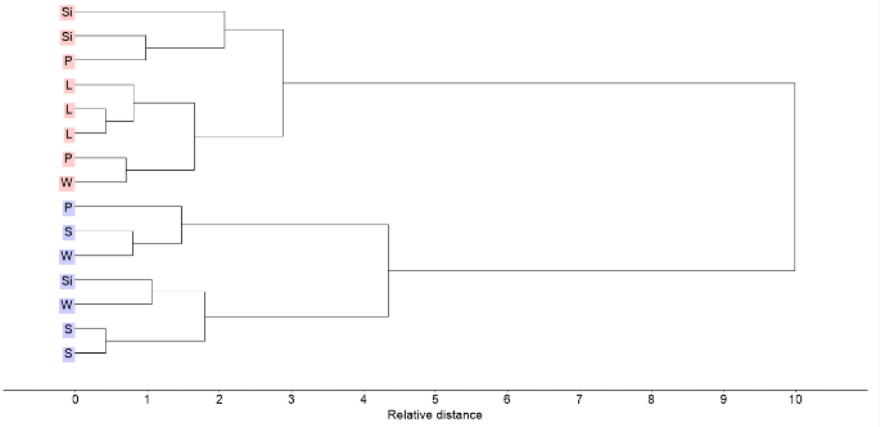
(b)

Figure 2.3. Univariate analysis of protein related amide region: (a) peak area of amide region; (b) peak high of α-helix with secondary derivative pre-treatment.

However, univariate analysis sometimes cannot differentiate the samples with selected peak height and area as only limited information contained in spectrum is used, in the meantime, multivariate analysis utilizes all information contained in the spectrum and overcome the shortage of univariate analysis. In addition, multivariate analysis has proved its capability in detecting inherent microstructure differences in synchrotron SR-IMS (Yu 2005a). There are two types of common multivariate analysis that have been utilized in spectral analysis, they are hierarchical cluster analysis (CLA) and principle component analysis (PCA) (Yu 2005a).



(a)



(b)

Figure 2.4. Example of results of faba bean spectral feature in lipid related region (a): Principle component analysis (PCA); (b): Hierarchical cluster analysis (CLA)

As for CLA, an agglomerative hierarchical analysis is used to present data as dendrograms. Specifically, two most similar IR spectra is searched and cluster into a new hierarchical group by calculating the distance matrix with algorithm, and the new hierarchical group will be treated as normal IR spectrum and do the distance matrix calculation again until all spectrum are clustered into the one general group.

The PCA however, aims to derive several uncorrelated variables (principal components) to explain as much as possible of variability in the original variables. Univariate and multivariate analysis are usually combined to differentiate spectral difference between the samples.

2.8. Literature summary, research objectives and hypotheses

2.8.1. Literature summary

In recent decades, the production of pulse crops in Canada has increased at an astounding rate (Bekkering 2011), and it is especially true in western Canada (Phelps 2017). Faba beans as a specie of pulses also seen great increase in its production due to the improvement of technology and the expansion of market. As a result, using faba beans to substitute some feeds ingredients shows its economic values. Faba bean exhibits its potential to be used as an alternative feeds resource. However, little information has been obtained about chemical and nutrient profiles, specific feed characteristics/bioactive compound, available nitrogen to energy synchronization, and true nutrient utilization and availability, especially for the currently developed varieties/lines of faba beans grown in western Canada. In addition, studies about faba bean have focused on faba bean seeds with very limited study about faba bean silage. As a result, nutritional study about faba bean

partitions and faba bean forage could give some insight about the nutritional availability and digestion characteristics of faba bean for ruminants.

Utilization of FTIR in feed research enables us to correlate its nutritional availability and digestion characteristics with molecular spectral features change. Rumen degradation is the most important and mysterious part of feed digestion. However, very limited study is available to figure out the change of spectral feature during rumen incubation and whether it is correlated with digestion and nutritional feature of feedstuff.

2.8.2. Research objectives

General:

Compare faba bean samples in the structural, physicochemical, and nutritional characteristics. Using different parts of faba bean, whole plant faba bean and whole plant faba silage as references to study the effect of rumen digestion on the change of its spectral structure and its association to protein and carbohydrate digestion and metabolism characteristics.

Long-term:

1. To efficiently utilize all faba beans partitions and whole plant faba silage as alternative feed in ruminant systems for improving animal production and performance.
2. To determine how change of faba feed spectral features in the rumen is associated with the digestion of carbohydrate and protein.

Short-term:

1. To systematically determine chemical and nutrient profiles of faba bean plants in terms of leaf, stem, whole pods, whole plant, and whole plant faba silage for dairy cows.

2. To compare the molecule structure spectral profiles of leaf, stem, whole pods, whole plant and whole plant faba silage in original samples and rumen digested residual samples.
3. To associate molecular structure spectral profiles to nutrient utilization and availability and quantify the relationship between molecular spectral profiles and nutrient utilization and availability in ruminants.

2.8.3. Research hypothesis

In general:

Leaf, stem, whole pods, whole plant, and whole plant faba silage could differ in structural, physicochemical, and nutritional characteristics. Structural change of faba feed in the rumen could be associated with its digestion and utilization.

In detail:

1. Utilization and availability could differ among leaf, stem, whole pods, whole plant and whole plant faba bean forage and whole pods faba bean could be used to feed ruminant (project 1).
2. Molecule structure spectral profiles could differ among leaf, stem, whole pods, whole plant and whole plant faba silage in original samples and rumen digested residual samples (project 2).
3. Molecular structures could have relationship with the nutrient utilization and availability and the relationship could also be found between original samples and rumen degradation as well as intestinal digestion in term of molecular spectral profiles (project 3).

3. CHEMICAL AND NUTRIENT PROFILES, N-TO-ENERGY SYNCHRONIZATION, RUMEN DEGRADATION AND INTESTINAL DIGESTION OF RECENTLY DEVELOPED FABA BEAN PLANTS: COMPARISON AMONG FABA LEAF, STEM, WHOLE PODS, WHOLE PLANT AND WHOLE PLANT FABA SILAGE.

3.1. Abstract

The objective of this study was to explore the potential to utilize different parts of faba bean as alternate feeding source for ruminants. Chemical compositions of different faba bean samples (faba bean leaf, stem, whole pods, whole plant, and whole plant faba silage) were determined. Based on the chemical compositions, energy values and CNCPS fractions were calculated. Afterwards, faba bean samples were incubated in the rumen for determination of ruminal degradation kinetics and energy to N synchronization. With 12 h rumen pre-incubation samples, the intestinal digestibility of protein was determined using three-step *in vitro* method. Lastly, metabolizable protein supply and feed milk value (FMV) were estimated with both NRC-2001 and DVE/OEB system. The experimental design used was CRD (different faba bean samples as fixed effect). Procedure MIXED of SAS 9.4 was used for statistical analysis with significant level declared as $P < 0.05$ and trends at $0.05 \leq P \leq 0.10$. According to the results, WP had the highest starch content, which was significantly higher than whole plant silage. Starch content of faba silage was significantly higher than WPL. As for protein profiles, WP faba bean had the highest CP, SCP and the lowest neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP). Faba bean silage had higher CP content than WPL. The similar trend was observed in CNCPS fractions and energy values. In terms of the metabolizable protein supply and feed milk value, in NRC-2001 system WPL, WP and silage were predicted to be comparable while in DVE/OEB system, WP was predicted to be higher than WPL and silage. Regarding feed milk

value, WPL, WP and faba bean silage were all predicted to be comparable or higher than some commonly used feed resource. Overall, faba bean WPL, WP and faba bean silage have potential to be used as feeding resource for ruminants.

3.2. Introduction

The growing world population has resulted in increasing interest of finding alternative feeding resources to meet people's need for livestock products, and faba bean (*Vicia faba* L.) which riches in high quality starch and protein content has showed its potential to fill the gap of such need (Duc et al. 1999; Warsame et al. 2018). In addition to be used as a protein source, the N-fixing ability of faba bean demonstrates its potential use in sustainable cropping system (Jensen et al. 2010). However, the application of faba bean to feed industry was limited because of the presence of antinutritional factors such as tannin (Crépon et al. 2010). With recent progress made in plant breeding, the genotype which contains about 1% of tannin appeared, after that, the planting areas of faba bean soared, and in Canada around 10 times of increase can be seen in the western province of Alberta from 2012 to 2015 (Olson n.d.).

Several studies have been conducted to test the feeding potential of faba bean (Grosjean et al. 2001; Volpelli, Luisa Antonella et al. 2009; Crépon et al. 2010), and recent studies have found it feasible to replace soybean meal with faba bean in dairy cows (Cherif et al. 2018; Mendowski et al. 2019). In addition, the use of whole plant faba bean silage as forage source has been tested and the potential was proved (McKnight and MacLeod 1977). However, since then no animal trial of feeding faba bean silage to ruminants can be found. Because dramatic improvement in faba bean breeding has been made, its chemical and nutritional characteristics could also have changed to a great extent. Therefore, the feeding values of faba bean silage should be determined again to prove its potential in the industry. In addition, studies concerning the nutritive values of faba bean have

concentrated on faba bean seeds with only one study compare basic chemical compositions of faba bean straw partitions (Alkhtib et al. 2016), no study has systematically determined the feed value of different partitions of faba bean. As a result, this study was conducted to evaluate the feeding values of different partitions of faba bean, whole plant faba bean and whole plant faba silage in terms of chemical compositions and nutritional availability.

3.3. Study objectives

The objectives of this study were to compare nutritional features and digestion characteristics of faba bean in ruminant among leaf, stem, whole pods, whole plant and whole plant faba bean silage.

3.4. Study hypotheses

The study hypothesis was that nutritional features and digestion characteristics could differ among different faba bean samples and faba bean could be used as forage resource for ruminant.

3.5. Materials and Methods

3.5.1. Sample information

The samples of whole plant, leaf, stem, and whole pods of the faba bean were harvested from three different plots (plot area equals 36 square feet with 180 seeds per plot) in, the research filed of the University of Saskatchewan. The average yield of low tannin faba bean at flower, mid pod and late pod stages was 65.90 tons/hectare as fresh matter base and not significant difference observed among different stages. However, on dry matter base significant higher yield was seen at late pod stage which was on average 12.20 tons/hectare for low and high tannin (not significant difference between the low and high tannin types). In addition, the preliminary study also proved the highest quality of faba bean to be used as forage at late pod stage of maturity, therefore faba bean sample at late pod stage with 97 days in maturity was used in this study. The whole plant faba bean used for making silage was harvested by machine while faba bean partitions and whole plant faba bean

were harvested manually. After harvesting the samples were wilted for two days and chopped to 1 -inch length. Whole plant faba silage was made with whole plant faba bean from three different plots using mini silos (three silos corresponding to three different plots) with around 2.5 kg of chopped whole plant faba bean (1 -inch length) per mini silo (10 cm in diameter and 40 cm in length). Faba bean silage was firmly packed and fermented for 120 days. In addition, faba bean silage was made with good quality (pH equals 4.5 and no observable mold). For each faba bean samples of each plots, multiple plants (or partitions of plants) were collected and mixed. Overall, 5 different faba bean samples (whole plant, stem, leaf, whole pods and faba silage) from three different plots (as replications) were collected for the present study (n=5×3). The variety of faba bean used for the study was CDC Snowdrop as it is the most common variety used in the province of Saskatchewan as feed resource, and all the samples were from the same year (2018).All samples were ground through a 1.0 mm screen (Retsch ZM 200, Retsch Inc, Haan, Germany) for detailed chemical profile analyses, a 3.0 mm screen for *in situ* rumen incubation and a 0.12 mm screen for molecular spectral analyses.

3.5.2. Determination of chemical profiles

The samples of faba bean partitions and faba silage were analyzed for dry matter (DM) (AOAC 930.15), ash (AOAC 942.05), crude protein (CP) (AOAC 984.13), ether extract (AOAC 920.39). Acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) were analyzed using ANKOM F57 filter bags (ANKOM Technology Corp., Fairport, NY), according to Van Soest et al. (1991). Cellulose (ADF - ADL) and hemicellulose (NDF - ADF) were calculated according to NRC (2001); With NDF and ADF residues, neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) were determined using CP procedure; soluble crude protein (SCP) was analyzed with the method described by Roe (1990); non-protein nitrogen

(NPN) was measured using procedures of Licitra et al. (1996) and starch was analyzed with total starch assay (AOAC method 966.11, AACC method 76.13, ICC standard method No.168).

3.5.3. Determination of protein and carbohydrate sub-fractions (CNCPS 6.5)

Protein and Carbohydrate Sub-fractions were described by CNCPS system 6.5 Version (Van Amburgh et al. 2015). Specifically, protein were partitioned as: PA1 (ammonia); PA2 (soluble true protein and fast degradable protein fraction); PB1 (intermediately degradable fraction of protein); PB2 (slowly degradable fraction of protein) and PC (undegradable protein fraction); while carbohydrate fractions were partitioned as: CA, fast degradable fraction of CHO (which include sub-fractions: CA1, short chain fatty acids; CA2, lactic acids; CA3, other organic acids; CA4, water soluble carbohydrates); CB1 (rapidly degradable fraction of CHO); CB2 (intermediate degradable fraction of CHO); CB3 (slowly degradable fraction of CHO) and CC (unavailable CHO fraction), which was estimated from 288 h incubation residues.

3.5.4. Determination of energy values

Total digestible nutrients, and digestible, metabolizable and net energy values for dairy and beef cattle were determined using the NRC summative approach (NRC 2001, 2016). The parameters include: gross energy (GE), total digestible crude protein (tdCP), total digestible NDF (tdNDF), total digestible non-fiber carbohydrate (tdNFC), total digestible fatty acid (tdFA), total digestible nutrient (TDN), digestive energy (DE), net energy for maintenance (NE_m), net energy for gain (NE_g), net energy for lactation (NE_L) at both maintenance and production levels.

3.5.5. Rumen degradation kinetics

Rumen Incubation Procedure:

There were four cannulated Holstein Friesian milking cows used for the *in situ* study at Rayner Dairy Teaching and Research Facility (University of Saskatchewan, Saskatoon, Canada). The

cows were kept in tie stalls during the period of sampling. Rumen incubation was conducted using the standard *in situ* rumen incubation procedure (Xin and Yu 2013b). Dry matter (DM), organic matter (OM), CP and NDF degradation characteristics were determined. Briefly, incubations in the rumen were carried with 7 g DM in each nylon bag. Nylon bags were incubated in the rumen with the 'gradual addition/all out' schedule. Specifically, samples were incubated in the rumen for 0, 3, 6, 12, 24, 48, 72 and 288 h. For each time point of 0, 3, 6 h, 2 bags were used; three bags were used for time points 12, 24 and 48 h; 4 bags were used for time point of 72 h to ensure enough samples left for analysis. A maximum of 32 bags were incubated in the rumen at each time point. After incubation, samples were taken out from the rumen and rinsed in cold water for 6 times to remove excess ruminal contents. After rinsing, the bags were placed at 55℃ oven for 48 h and recorded for weight.

Rumen Degradation Kinetics:

With the results of *in situ* incubation, rumen degradation kinetic parameters were determined according to the equation described by published papers (Ørskov and McDonald 1979; Tamminga et al. 1994). The equation is described as: $R(t) = U + D \times e^{-Kd\times(t-T0)}$, where R(t) (%) stands for residue after t hours' incubation; U equals undegradable fraction (%); D is potential degradable fraction (%); T0 stands for lag time (h); and K_d stands for degradation rate (% h^{-1}). With equation above, the effective degradability (ED) of each nutrient was predicted according to NRC (2001) as $ED\ (\%) = S + D \times K_d/(K_p + K_d)$, accordingly, S equals to soluble fraction (%); k_p stands for estimated rate of outflow from rumen (% h^{-1}) and is assumed to be 4.5% h^{-1} (Tamminga et al. 1994). Based on the results, total rumen degradable and undegraded protein contents were determined.

3.5.6. Intestinal digestion of protein

According to published papers (Calsamiglia and Stern 1995a; Gargallo and Calsamiglia 2006), the estimation of intestinal digestibility of rumen undegradable protein was conducted by a modified three-step in vitro method with the residue samples of 12 h pre-incubation. Firstly, 10 mL of pepsin (Sigma P-7012) solution (in 0.1 N HCl with pH 1.9) was added to residue samples, solution was then vortexed and incubated at 38 ℃ water bath for 1 h. After that, mixed solution was added with 0.5 mL 1 N NaOH solution and 13.5 mL of pancreatin (Sigma P- 7545), vortexed and incubated at 38 ℃ for 24 h, followed by addition of 3 mL of tri-carboxylic acid (TCA) to stop the enzymatic hydrolysis. Solution was then vortexed and centrifuged for 15 min at 10, 000 g, and supernatant (5 mL) was analyzed for soluble N by the Kjeldahl method. Protein intestinal digestibility was calculated as TCA-soluble N/ N in rumen residue sample.

3.5.7. Hourly effective rumen degradation ratios /potential N-to-energy synchronization

Hourly effective rumen degradation ratio was calculated based on results of rumen incubation kinetics. According to Sinclair et al. (1993), the effective extent OM and N that degraded every hour was calculated every hour by followed equation: Hourly ED (g kg^{-1} DM) = S+ $[(D\times K_d)/(K_p + K_d)]\times 1- e^{-t\times(Kd+Kp)}$. According to quantity of OM and N degraded every hour, an hourly ratio of N to OM was determined as: Hourly ED ratio N/OMt = (HEDNt - HEDNt-1)/(HEDOMt - HEDOMt-1), where N/OMt equals to ratio of N to OM at time t (g N kg^{-1} OM); HEDNt equals to hourly effective degradability of N at time t (g kg^{-1} DM); HEDNt-1 equals to hourly effective degradability of N 1 hour before t (g kg^{-1}DM); HEDOMt equals to hourly effective degradability of OM at time t (g kg^{-1} DM); HEDOMt-1 = hourly effective degradability of OM 1 hour before t (g kg^{-1} DM).

3.5.8. Prediction of nutrient supply using DVE/OEB and NRC Dairy 2001 systems

Metabolizable protein of faba bean samples were estimated using DVE/OEB system (Tamminga et al. 1994) and the NRC-2001 system (NRC 2001) to predict the metabolizable protein supply. In addition, feed milk value (FMV) was estimated to predict the potential of milk production per kg feed. In both systems, the metabolizable protein have three sources, absorbable microbial protein, endogenous true protein and absorbable rumen undegradable feed protein, while microbial fraction was estimated from both available energy and available protein. The difference of microbial protein from available energy and available protein was also calculated. Different equations and naming methods are used in two systems, which are detailly described in Theodoridou and Yu (2013a). Specifically, DVE/OEB system constitutes of two parts, the DVE and the OEB value. The DVE value is constituted of digestible feed protein, microbial protein, and an endogenous protein loss correction while the equation is described as : $DVE = AMCP^{DVE} + ARUP^{DVE} – ENDP$, where $AMCP^{DVE}$ is the microbial crude protein that is absorbable; $ARUP^{DVE}$ is the ruminally undegradable feed protein and ENDP is used for correcting the endogenous protein lost during the digestion process. The OEB value is described as equation $OEB = MCP_{RDP}^{DVE} - MCP_{FOM}$. MCP_{RDP}^{DVE} is the potential MCP synthesis based on RDP. MCP_{FOM} is potential MCP synthesis using energy from anaerobic fermentation. The MCP_{RDP}^{DVE} is calculated as equation $MCP_{RDP}^{DVE} = CP \times [1 - (1.11 \times RUP (\% CP)/100)]$.

Similar with DVE/OEB system, the metabolizable protein supply in NRC-2001 model has the same sources. However, different with DVE/OEB system, the endogenous protein is contributed to the metabolizable protein. According to NRC-2001 model (NRC 2001), the calculation is given as below. When RDP exceed 1.18 ×TDN-predicted MCP (MCP_{TDN}), MCP_{TDN} (Potential ruminally synthesized microbial CP) will be calculated as MCP_{TDN} (g/kg of DM) =0.13 × TDN (discounted), When RDP is less than 1.18 × TDN-predicted MCP (MCP_{TDN}), then MCP will be calculated as

0.85 of RDP (MCP_{RDP}^{NRC}); truly absorbed MCP ($AMCP^{NRC}$) will be calculated as $AMCP^{NRC}$ (g/kg of DM) = 0.80 × 0.80 × MCP_{TDN}, where true protein and digestibility of ruminally synthesized microbial CP are both defined as 800g/kg in NRC-2001; $ARUP^{NRC}$ (Truly absorbed rumen-undegraded protein in the small intestine) will be calculated as $ARUP^{NRC}$ = RUP^{NRC} × dRUP (intestinal digestibility of rumen undegraded protein); rumen endogenous protein in the small intestine (ECP^{NRC}) will be calculated as ECP (g/kg of DM) = 6.25 × 1.9 × DM; truly absorbed rumen endogenous protein in the small intestine (AECP) value will be calculated as AECP (g/kg of DM) = 0.50 × 0.80 × ECP; total metabolizable protein (MP) will be calculated as MP (g/kg of DM) =$ARUP^{NRC}$ + $AMCP^{NRC}$ + AECP; the degraded protein balance (DPB^{NRC}), which describe the difference between potential microbial protein synthesize based on energy RDP, will be calculated as DPB^{NRC} (g/kg of DM) = RDP^{NRC} - 1.18 ×MCP_{TDN}.

3.5.9. Statistical analyses

Procedure MIXED of SAS 9.4 software (SAS Institute, Inc., Cary, NC, USA) was performed for statistical analyses. Completely random design (CRD) was used in the study. The used model was $Y_{ij} = Mean + Trt_i + Error_{ij}$, where Y_{ij} was the observation of the independent variable; Mean was the population mean of the variable; Trt_i was the fixed effect of treatments (i=5; leaf, whole pods, stem, whole plant and whole plant silage); $Error_{ij}$ was the random error associated with the observation. Model assumption was checked by Residual Analysis using Proc Univariate with Normal and Plot options. Multi-comparisons were conducted with Tukey-Kramer method. Normality test of residual data were conducted with Proc univariate using Shapiro-Wilk method. For all statistical analyses, significance was declared at $P < 0.05$ and trends at $0.05 \leq P \leq 0.10$. The NLIN (nonlinear) procedure of SAS was used to analyze results with iterative least squares regression (Dhanoa 1988).

3.6. Results and Discussion

3.6.1. Chemical and nutrient profiles of recently developed faba bean plants: comparison among faba bean leaf, stem, whole pods, whole plant and whole plant silage

The basic chemical profiles of different faba bean partitions and faba bean forage are presented in Table 3.1. According to the results, faba bean partitions had significantly different chemical content ($P < 0.001$). Stem of faba bean had the highest NDF, ADF and ADL contents, which shows the lowest nutritive value of faba bean stem. However, the values of NDF, ADF and ADL contents of stem is this study were lower than the results in the study of Alkhtib et al. (2016), which may because of variety difference. NDF, ether extract and CP contents of faba bean leaf in this study were comparable with brassica plant, according to the results of Barry (2013). Specifically, faba bean leaf had 37 g kg^{-1} DM of ether extract compared with 11 to 36 g kg^{-1} DM in brassica plants; NDF content of faba bean leaf was 247 g kg^{-1} DM, while that of brassica plants was around 200 g kg^{-1} DM; CP content was also similar between faba bean leaf and brassica plants. The NDF and ADF contents of faba bean whole plants were 353 and 269 g kg^{-1} DM respectively, which were much lower than the results of Mustafa and Seguin (2003) (457 and 305 g kg^{-1} DM respectively). In addition, starch content of WPL in this study was much higher than that of Mustafa and Seguin (2003) (137 compared with 29 g kg^{-1} DM). According to the study of Rahman et al. (2019) the chemical difference of faba bean seeds between varieties was relatively small, however large variance may still exist in chemical content of whole plant between different faba bean varieties.

As for protein related chemical features, whole pods (WP) had the highest CP (271 g/kg DM) and considerable proportion was SCP (199 g/kg DM). The lowest ADICP content (0.87 g/kg DM) of WP was also observed, which shows the highest nutritional value of WP. Faba bean silage compared with whole plant faba bean (WPL), had higher CP content and lower NDICP content

(212 vs 187 g/kg DM and 7.9 vs 12.9 g/kg DM respectively, $P < 0.001$). The CP protein content of faba silage was comparable with other study (Mustafa and Seguin 2003) and was higher than other commonly used silage sources (Mustafa et al. 2000; Beauchemin and McGinn 2005). The CP protein content of faba bean leaf was comparable with WPL and was much higher than stem (192 vs 49 g/kg DM, $P < 0.001$), however, it also had the highest NDICP and ADICP contents (29 and 9 g/kg DM respectively). It is noticeable that the starch and CP contents in faba bean silage were higher than that in faba bean whole plant. Normally, after fermentation the starch and CP contents in silage are low than before ensiling. However, the increased content of CP and starch may because the fermentation process decrease the total biomass and make the final starch and CP content on dry matter base higher than before ensiling. Different harvest methods of whole plant faba bean silage and whole plant faba bean may also account for part of the reason, specifically, manual harvest of whole plant may cause lose of pods which higher in starch and CP while machine harvest maintaining whole biomass to a greater extent.

In conclusion, the whole plant faba silage had comparable NDF and ADF contents, and higher starch and CP contents than commonly used pea, barley and alfalfa silage (Mustafa et al. 2000), which indicates the potential of faba silage as alternative silage resource. Whole pods of faba bean, which had the considerable nutrients profiles could also be used as alternate feeding resource for ruminants because rumen is capable of degrading major antinutritional factors in seed and hull of faba bean. However, animal trials of feeding faba bean whole pods and whole plant silage to cattle are needed to test the feeding potential of whole plant faba silage.

Table 3.1. Chemical and nutrient profiles of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage.

	Faba bean partitions						
Parameter	WPL	Stem	Leaf	WP	Silage	SEM	*P* value
% Whole plant		27.7	6.0	66.3			
DM (g/kg)	928^{c}	939^{b}	921^{d}	925^{c}	952^{a}	0.9	<0.001
Ash (g/kg DM)	73^{bc}	86^{b}	154^{a}	50^{d}	69^{c}	3.2	<0.001
EE (g/kg DM)	13^{b}	3^{c}	37^{a}	7^{bc}	13^{b}	1.7	<0.001
NDF (g/kg DM)	353^{b}	623^{a}	248^{c}	215^{c}	328^{b}	10.4	<0.001
ADF (g/kg DM)	270^{b}	515^{a}	202^{c}	156^{d}	264^{b}	9.6	<0.001
ADL (g/kg DM)	42^{c}	95^{a}	60^{b}	15^{d}	36^{c}	2.5	<0.001
Starch (g/kg DM)	137^{c}	11^{d}	6^{d}	261^{a}	176^{b}	8.2	<0.001
Sugar (g/kg DM)	47^{b}	44^{b}	78^{a}	42^{b}	8^{c}	3.6	<0.001
NDICP (g/kg DM)	13^{b}	7^{c}	29^{a}	13^{b}	8^{c}	0.5	<0.001
ADICP (g/kg DM)	2^{bc}	3^{b}	9^{a}	1^{c}	2^{bc}	0.3	<0.001
SCP (g/kg DM)	122^{b}	27^{d}	95^{c}	200^{a}	128^{b}	4.4	<0.001
CP (g/kg DM)	187^{c}	49^{d}	192^{c}	272^{a}	212^{b}	3.5	<0.001

Notes: $^{a\text{-}d}$ Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparison using Tukey method. WPL, whole plant; WP, whole pods; DM: dry matter; EE: ether extract (crude fat); NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; CP: crude protein.

3.6.2. Energy profiles of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage

The results of energy profiles of faba bean partitions and faba bean forage are shown in Table 3.2. According to the results, WP had the highest truly digestible non-fiber carbohydrate (tdNFC) value (46.03 % of DM) while WPL and faba silage had similar results of tdNFC. With regard to truly digestible crude protein (tdCP), WP also had the highest value (27.1 % of DM), followed by faba bean silage (21.0 % DM) and then WPL (18.5 % DM). As for energy values of total digestible nutrient at one time maintenance ($TDN_{1\times}$); total digestible nutrient at production level of intake(3×) ($TDN_{3\times}$); digestible energy at one time maintenance ($DE_{1\times}$); digestible energy at production level of intake (3×) ($DE_{3\times}$); metabolizable energy at production level of intake (3×) (ME_{3x}); net energy for lactation at production level of intake (3×) ($NE_{L3\times}$); net energy for maintenance (NE_m) and net energy for growth(NE_g), same trend was observed, which was highest for WP, secondary for WPL and faba silage followed by leaf and stem had the lowest energy values.

Table 3.2. Energy profiles of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage.

Energy profiles	Faba bean partitions					SEM	*P* value
	WPL	Stem	Leaf	WP	Silage		
tdNFC (% DM)	37.87^{b}	22.22^{c}	38.99^{b}	46.03^{a}	37.82^{b}	0.879	<0.001
tdCP (% DM)	18.49^{c}	4.55^{d}	18.11^{c}	27.06^{a}	20.99^{b}	0.361	<0.001
tdFA (% DM)	0.37^{b}	0.00^{b}	2.73^{a}	0.02^{b}	0.28^{b}	0.143	<0.001
tdNDF (% DM)	16.84^{b}	29.15^{a}	6.97^{d}	11.53^{c}	16.37^{b}	0.517	<0.001
TDN_{1x} (% DM)	67.03^{b}	48.92^{d}	63.20^{c}	77.68^{a}	68.80^{b}	0.755	<0.001
TDN_{3x} (% DM)	63.50^{b}	48.92^{d}	61.19^{c}	70.32^{a}	64.63^{b}	0.464	<0.001
DE_{1x} (Mcal/kg)	3.07^{b}	2.11^{d}	2.90^{c}	3.64^{a}	3.18^{b}	0.035	<0.001
DE_{3x} (Mcal/kg)	2.91^{b}	2.11^{d}	2.81^{c}	3.29^{a}	2.98^{b}	0.022	<0.001
NE_{L1x} (Mcal/kg)	1.67^{b}	0.99^{d}	1.56^{c}	2.08^{a}	1.75^{b}	0.025	<0.001
NE_{L3x} (Mcal/kg)	1.56^{b}	0.99^{c}	1.55^{b}	1.83^{a}	1.61^{b}	0.019	<0.001
ME (Mcal/kg)	2.65^{b}	1.68^{d}	2.49^{c}	3.22^{a}	2.76^{b}	0.035	<0.001
ME_{3x} (Mcal/kg)	2.49^{b}	1.68^{c}	2.47^{b}	2.88^{a}	2.56^{b}	0.026	<0.001
NE_{m} (Mcal/kg)	1.62^{b}	0.90^{d}	1.50^{c}	2.02^{a}	1.70^{b}	0.025	<0.001
NE_{g} (Mcal/kg)	1.01^{b}	0.35^{d}	0.91^{c}	1.36^{a}	1.08^{b}	0.022	<0.001

Notes: $^{a-d}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; tdNFC, truly digestible nonfiber carbohydrate; tdCP, truly digestible crude protein; tdFA, truly digestible fatty acid; tdNDF, truly digestible neutral detergent fiber; TDN1x, total digestible nutrient at one time maintenance; TDN_{3x}, total digestible nutrient at production level of intake(3×); DE_{1x}, digestible energy at one time maintenance estimated from NRC dairy model 2001; $NE_{L1x,}$ net energy for lactation at one time maintenance; DE_{3x}, digestible energy at production level of intake(3×) estimated from NRC dairy model 2001; ME_{3x}, metabolizable energy at production level of intake (3×) estimated from NRC dairy model 2001; NE_{L3x}, net energy for lactation at production level of intake (3×) estimated from NRC dairy model 2001; NE_{m}, net energy for maintenance estimated from NRC beef model 1996; NE_{g}, net energy for growth estimated from NRC beef model 1996.

3.6.3. Partitioning protein fractions and carbohydrate fractions in recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage

The results of carbohydrate and protein partitioning and predicted degradable and undegradable fractions of faba bean samples according to CNCPS system is showed in Tables 3.3 and 3.4 respectively. PA2 fraction, which is readily degradable and easily degraded to NH_3-N in the rumen, took the majority part of CP in WP, WPL and faba silage, and was the highest in the whole pods of faba bean and may cause rumen bloat when feeding to cattle with large amount. However, the carbohydrate fractions of WPL and faba silage were mainly CB2 and CB3 fractions which are intermediate degradable. As a result, to acquire maximum efficiency of N utilization, the use of WPL, WP and faba silage as protein source should coordinate with more readily degradable carbohydrate resources. WP of faba bean had minimal fraction of PC which shows the protein content of WP is of good quality. Faba bean silage compared with whole plant faba bean, had much lower CA4 fraction and higher CB1 fraction. In addition, rumen degradable total crude protein of whole plant faba silage was significantly higher than whole plant faba bean ($P < 0.001$) with rumen degradable total carbohydrate numerically higher than whole plant faba bean, which proves the ensiling potential of whole plant faba bean. Stem compared with other faba bean partitions had the highest rumen undegradable CP although it had the lowest CP content, and the highest rumen undegradable carbohydrate, thus having minimal nutritive value. Leaf of faba bean had high quality carbohydrate and protein sources, which reflected at the highest PB and CA4 (water soluble carbohydrates) fractions. In addition, leaf of faba bean had comparable rumen degradable carbohydrate with WP and the least rumen undegradable crude protein.

Table 3.3. Partitioning protein fractions and carbohydrate fractions in recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage.

	Faba bean partitions						
Item	WPL	Stem	Leaf	WP	Silage	SEM	*P* value
Protein sub-fractions							
PA1 (g/kg DM)					2		
PA2 (g/kg DM)	122^{b}	27^{d}	95^{c}	200^{a}	127^{b}	4.3	<0.001
PB1 (g/kg DM)	56^{b}	21^{c}	86^{a}	60^{b}	80^{a}	4.1	<0.001
PB2 (g/kg DM)	11^{b}	4^{c}	20^{a}	12^{b}	6^{c}	0.5	<0.001
PC (g/kg DM)	2^{bc}	3^{b}	9^{a}	1^{c}	2^{bc}	0.3	<0.001
PA2 (% of true protein)	0.65^{b}	0.52^{cd}	0.47^{d}	0.73^{a}	0.60^{bc}	0.02	<0.001
PB1 (% of true protein)	0.30^{bc}	0.40^{a}	0.43^{a}	0.22^{c}	0.38^{ab}	0.02	<0.001
PB2 (% of true protein)	0.058^{bc}	0.080^{ab}	0.098^{a}	0.045^{cd}	0.028^{d}	0.005	<0.001
Carbohydrate sub-fractions							
CA1 (g/kg DM)					5		
CA4 (g/kg DM)	47^{b}	44^{b}	78^{a}	42^{b}	8^{c}	3.6	<0.001
CB1 (g/kg DM)	137^{c}	11^{d}	6^{d}	261^{a}	176^{b}	8.1	<0.001
CB2 (g/kg DM)	196^{b}	171^{bc}	290^{a}	157^{c}	193^{b}	7.8	<0.001
CB3 (g/kg DM)	208^{b}	279^{a}	163^{c}	183^{bc}	210^{b}	9.4	<0.001
CC (g/kg DM)	134^{b}	367^{a}	80^{c}	28^{d}	122^{b}	8.1	<0.001

Notes: $^{a-d}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; PA1, rapidly degradable protein fraction (ammonia); PA2, rapidly degradable protein fraction (soluble true protein); PB1, intermediately degradable protein fraction (insoluble true protein); PB2, rapidly degradable protein fraction (fiber-bound protein); PC, indigestible protein or unavailable protein fraction; CA1, rapidly degradable carbohydrate fraction (volatile fatty acids); CA4, rapidly degradable carbohydrate fraction (water soluble carbohydrate); CB1, intermediately degradable carbohydrate fraction (starch); CB2, intermediately degradable carbohydrate fraction (soluble fiber); CB3, intermediately degradable carbohydrate fraction (digestible fiber); CC, unavailable neutral detergent fiber (indigestible fiber).

Table 3.4. Degradable and digestible content of each fraction in protein and carbohydrates of recently developed faba bean plants in rumen phase and intestinal phase: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage.

	Faba bean partitions						
Item	WPL	Stem	Leaf	WP	Silage	SEM	*P* value
Predicted RDC							
RDCA4 (% of DM)	3.80^{b}	3.95^{b}	7.02^{a}	3.81^{b}	0.65^{c}	0.327	<0.001
RDCB1 (% of DM)	11.92^{c}	0.98^{d}	0.53^{d}	22.14^{a}	15.28^{b}	0.711	<0001
RDCB2 (% of DM)	15.97^{b}	14.84^{b}	25.67^{a}	13.67^{b}	15.75^{b}	0.6646	<0.001
RDCB3 (% of DM)	15.96^{ab}	18.94^{a}	10.87^{ab}	9.64^{b}	17.56^{ab}	1.949	<0.001
RDCHO (% of DM)	47.65^{a}	38.71^{b}	44.09^{ab}	49.26^{a}	49.24^{a}	2.021	<0.001
Predicted RUC							
RUCA4 (% of DM)	0.86^{a}	0.45^{b}	0.79^{a}	0.43^{b}	0.15^{c}	0.037	<0.001
RUCB1 (% of DM)	1.79^{c}	0.15^{d}	0.08^{d}	3.95^{a}	2.29^{b}	0.108	<0.001
RUCB2 (% of DM)	3.59^{a}	2.23^{b}	3.30^{a}	2.05^{b}	3.54^{a}	0.125	<0.001
RUCB3 (% of DM)	7.93^{b}	14.41^{a}	5.44^{c}	8.62^{b}	8.01^{b}	0.323	<0.001
RUCHO	16.07^{ab}	23.11^{a}	9.61^{b}	15.04^{b}	16.80^{ab}	1.827	<0.01
Predicted RDP							
RDPA1 (% of DM)	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.17^{a}	0.003	<0.001
RDPA2 (% of DM)	10.81^{b}	2.45^{d}	8.29^{c}	17.15^{a}	11.26^{b}	0.384	<0.001
RDPB1 (% of DM)	2.93^{c}	1.59^{d}	6.28^{a}	4.41^{b}	4.22^{b}	0.239	<0.001
RDPB2 (% of DM)	0.69^{b}	0.20^{d}	1.33^{a}	0.66^{b}	0.36^{c}	0.034	<0.001
RDCP (% of DM)	14.44^{c}	4.23^{d}	15.90^{b}	22.21^{a}	16.00^{b}	0.292	<0.001
Predicted RUP							
RUPA2 (% of DM)	1.35^{bc}	0.22^{d}	1.18^{c}	2.82^{a}	1.41^{b}	0.051	<0.001
RUPB1 (% of DM)	2.64^{b}	0.48^{d}	2.36^{bc}	1.64^{c}	3.80^{a}	0.184	<0.001
RUPB2 (% of DM)	0.43^{b}	0.21^{c}	0.66^{a}	0.59^{a}	0.23^{c}	0.022	<0.001
RUCP (% of DM)	4.43^{bc}	0.91^{d}	4.20^{c}	5.05^{ab}	5.43^{a}	0.151	<0.001

Notes: $^{a-d}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; RDC, rumen degradable carbohydrate fractions; RUC, rumen undegradable carbohydrate fractions; RDP, rumen degradable protein fractions; RUP, rumen undegradable protein fractions; PA1, rapidly degradable protein fraction (ammonia); PA2, rapidly degradable protein fraction (soluble true protein); PB1, intermediately degradable protein fraction (insoluble true protein); PB2, rapidly degradable protein fraction (fiber-bound protein); PC, indigestible protein or unavailable protein fraction; CA1, rapidly degradable carbohydrate fraction (volatile fatty acids); CA4, rapidly degradable carbohydrate fraction (water soluble carbohydrate); CB1, intermediately degradable carbohydrate fraction (starch); CB2, intermediately degradable carbohydrate fraction (soluble fiber); CB3, intermediately degradable carbohydrate fraction (digestible fiber); CC, unavailable neutral detergent fiber (indigestible fiber).

3.6.4. Rumen degradation kinetics of recently developed faba bean plants: comparison among faba leaf, stem, pods, whole plant and whole plant silage

In addition to information of the basic chemical compositions and nutrients availability with various degradation rates, the kinetics of nutrients in the rumen are also of great value as efficient rumen fermentation requires both sufficient nutrient supply and coordination between energy and nitrogen. As a result, the rumen degradation kinetics of NDF, DM and OM were performed, and results are shown in Tables 3.5 and 3.6.

According to NDF degradation kinetics, leaf and WPL had the highest degradable fraction of NDF, followed by WP and faba silage. Although WP had the highest degradable fraction of NDF, it had similar effective degradable NDF with leaf and WPL, which were higher than faba silage.

Similar tendency was observed for OM and DM degradation kinetics. Generally, WP had the highest effective degradable DM and OM, followed by WPL and then faba silage; stem of faba bean had the least effective degradable fractions of DM and OM.

As for CP degradation kinetics, stem was excluded from the model as significant attachment and predation of microorganisms in the stem make it hard to count on its protein of plant origin. WP and leaf were not significantly different for degradation rate. But faba silage had significantly higher degradation rate than WPL (32.40 % h compared with 18.87 %/ h). The effective degradable crude protein (EDCP) was the highest in WP, which was higher than WPL and silage, while leaf had the least EDCP.

Table 3.5. Rumen degradation kinetics of neutral detergent fiber (NDF), dry matter (DM) and organic matter (OM) of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage.

	Faba bean partitions						
Item	WPL	Stem	Leaf	WP	Silage	SEM	*P* value
NDF degradation							
K_d (%/h)	8.59^{a}	3.58^{c}	8.38^{a}	4.55^{b}	4.41^{b}	1.110	0.023
T0 (h)	0.00	2.68	0.14	1.06	0.83	1.046	0.431
D (%)	60.70^{b}	37.83^{c}	67.40^{ab}	80.58^{a}	55.97^{bc}	3.962	<0.001
U (%)	39.30^{b}	62.17^{a}	32.60^{bc}	19.42^{c}	44.03^{ab}	3.962	<0.001
EDNDF (%)	38.75^{a}	16.55^{b}	43.84^{a}	39.07^{a}	25.08^{b}	1.901	<0.001
RUNDF (%)	61.25^{b}	83.45^{a}	56.16^{b}	60.93^{b}	74.92^{a}	1.901	<0.001
DM degradation							
K_d (%/h)	14.88^{bc}	12.66^{c}	24.37^{a}	14.86^{bc}	16.24^{b}	0.557	<0.001
T0 (h)	0.00	0.00	0.00	0.00	0.00		
S (%)	34.09^{ab}	18.89^{c}	43.03^{a}	34.09^{ab}	23.91^{bc}	2.942	0.001
D (%)	46.13^{ab}	25.72^{c}	39.96^{b}	53..99^{a}	51.74^{ab}	2.660	0.001
U (%)	19.77^{c}	55.39^{a}	17.01^{c}	10.67^{d}	24.35^{b}	0.742	<0.001
EDDM (%)	69.49^{b}	37.81^{d}	76.76^{a}	76.75^{a}	64.42^{c}	0.984	<0.001
RUDM (%)	30.51^{c}	62.19^{a}	23.24^{d}	23.25^{d}	35.58^{b}	0.984	<0.001
OM degradation							
K_d (%/h)	12.82^{b}	8.16^{c}	19.70^{a}	13.38^{b}	12.82^{b}	0.417	<0.001
T0 (h)	0.00	0.00	0.00	0.00	0.00		
S (%)	29.62^{ab}	11.64^{c}	36.25^{a}	32.26^{a}	15.89^{bc}	3.263	0.001
D (%)	50.67^{a}	30.72^{b}	46.13^{a}	57.48^{a}	59.27^{a}	2.910	<0.001
U (%)	19.71^{c}	57.64^{a}	17.63^{c}	10.25^{d}	24.84^{b}	0.845	<0.001
EDOM (%)	67.02^{b}	31.39^{d}	73.80^{a}	75.27^{a}	59.74^{c}	1.278	<0.001
RUOM (%)	32.98^{c}	68.61^{a}	26.20^{d}	24.73^{d}	40.26^{b}	1.278	<0.001

Notes: $^{a-c}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; NDF, neutral detergent fiber; DM, dry matter; OM, organic matter; K_d, rate of degradation of D fraction (%/h); T0, lag time; S, soluble fraction in the *in situ* incubation; D, potential degradable fraction; U, rumen undegradable fraction; EDNDF, effective degraded NDF; EDDM, effective degraded DM; EDOM, effective degraded OM; RUNDF, rumen undegraded feed NDF; RUDM, rumen undegraded feed DM; RUOM, rumen undegraded feed OM.

Table 3.6. Rumen degradation kinetics of crude protein (CP) of recently developed faba bean plants: comparison among faba leaf, whole pods, whole plant and whole plant silage.

	Faba bean partitions					
Item	WPL	Leaf	WP	Silage	SEM	*P* value
CP degradation						
K_d (%/h)	18.87^{c}	27.29^{b}	27.74^{b}	32.40^{a}	1.060	0.001
T0 (h)	0.00	0.00	0.00	0.00		
S (%)	49.45^{b}	48.15^{b}	58.94^{a}	54.88^{a}	0.941	0.001
D (%)	42.42^{a}	38.56^{ab}	36.18^{bc}	33.72^{c}	0.922	<0.001
U (%)	8.13^{b}	13.29^{a}	4.88^{c}	11.41^{a}	0.443	<0.001
EDCP (%)	83.65^{b}	81.25^{c}	89.99^{a}	84.47^{b}	0.474	<0.001
RUCP (%)	16.35^{b}	18.75^{a}	10.01^{c}	15.53^{b}	0.474	<0.001

Notes: $^{a-d}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; CP, crude protein; K_d, rate of degradation of D fraction (%/h); T0, lag time; S, soluble fraction in the *in situ* incubation; D, potential degradable fraction; U, rumen undegradable fraction; EDCP, effective degraded CP; RUCP, rumen undegraded feed CP.

3.6.5. N-to-energy synchronization of recently developed faba bean plants: comparison among faba leaf, whole pods, whole plant and whole plant silage

To optimize feed efficiency by maximizing microbial protein production, energy and protein releasing rate should be attached to the greatest importance (Clark et al. 1992). According to Nuez-Ortín and Yu (2010), it is important to acquire synchronization between energy availability and protein degradation. In detail, microbial protein synthesis can be maximize when 25 g N kg^{-1} OM is truly digested in the rumen (Nuez-Ortín and Yu 2010). To reflect the energy and protein releasing rate of faba bean samples, the results of rumen degradation kinetics and N to energy synchronization are showed in Table 3.7 and Figure 3.1 respectively. Stem had large proportion of cell wall content and underwent continuous microbial attachment and predation. Therefore, the weight change of stem during incubation can be influenced by the amount of firmly attached microbial fraction. As a result, stem is removed from the model of N to energy synchronization. According to the results, the N/OM value of silage and WP were significantly higher than WPL and stem ($P < 0.001$) and the release of N of silage and WP were dramatically decreased during first two hours. After 10 h, the N/OM value of WPL and leaf were significantly lower than silage and WPL ($P < 0.001$). As a result, the releasing pattern of N and energy in silage and WPL were unbalanced and should be cooperated with more readily digestible carbohydrate sources were feeding to cattle.

Table 3.7. Potentially available N to available OM synchronization of recently developed faba bean plants: comparison among faba leaf, whole pods, whole plant and silage.

	Faba bean partitions					
Item	WPL	Leaf	WP	Silage	SEM	*P* value
N/OM (g/kg)	32.33^{c}	36.00^{bc}	46.00^{a}	36.67^{b}	0.898	<0.001
ED_N/ED_OM(g/kg)	40.33^{b}	40.00^{b}	54.67^{a}	51.33^{a}	1.291	<0.001
Hourly effective degradation ratios of N to OM at individual times (g/kg)						
h0	54.00^{b}	48.33^{b}	84.00^{a}	92.00^{a}	4.328	<0.001
h1	38.67^{b}	40.33^{b}	55.00^{a}	48.00^{ab}	2.192	0.003
h2	36.67^{b}	37.67^{b}	47.67^{a}	39.67^{ab}	1.878	0.012
h3	34.33^{ab}	34.67^{ab}	41.67^{a}	32.67^{b}	1.740	0.027
h4	32.33^{ab}	32.33^{ab}	36.33^{a}	27.00^{b}	1.633	0.024
h6	28.67^{a}	27.67^{a}	27.00^{a}	18.33^{b}	1.384	0.003
h8	25.33^{a}	24.00^{a}	20.67^{a}	12.67^{b}	1.225	<0.001
h10	22.00^{a}	20.67^{a}	15.67^{b}	8.67^{c}	0.957	<0.001
h12	19.67^{a}	17.67^{a}	12.00^{b}	6.00^{c}	0.986	<0.001
h16	15.33^{a}	12.67^{a}	6.67^{b}	3.00^{b}	0.866	<0.001
h20	12.00^{a}	9.67^{a}	4.00^{b}	1.33^{b}	0.898	<0.001
h24	10.00^{a}	7.33^{a}	2.33^{b}	0.67^{b}	0.817	<0.001
h32	6.33^{a}	4.00^{a}	0.67^{b}	0.00^{b}	0.624	<0.001

Notes: $^{a-c}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; ED, effective degradability; OM, organic matters.

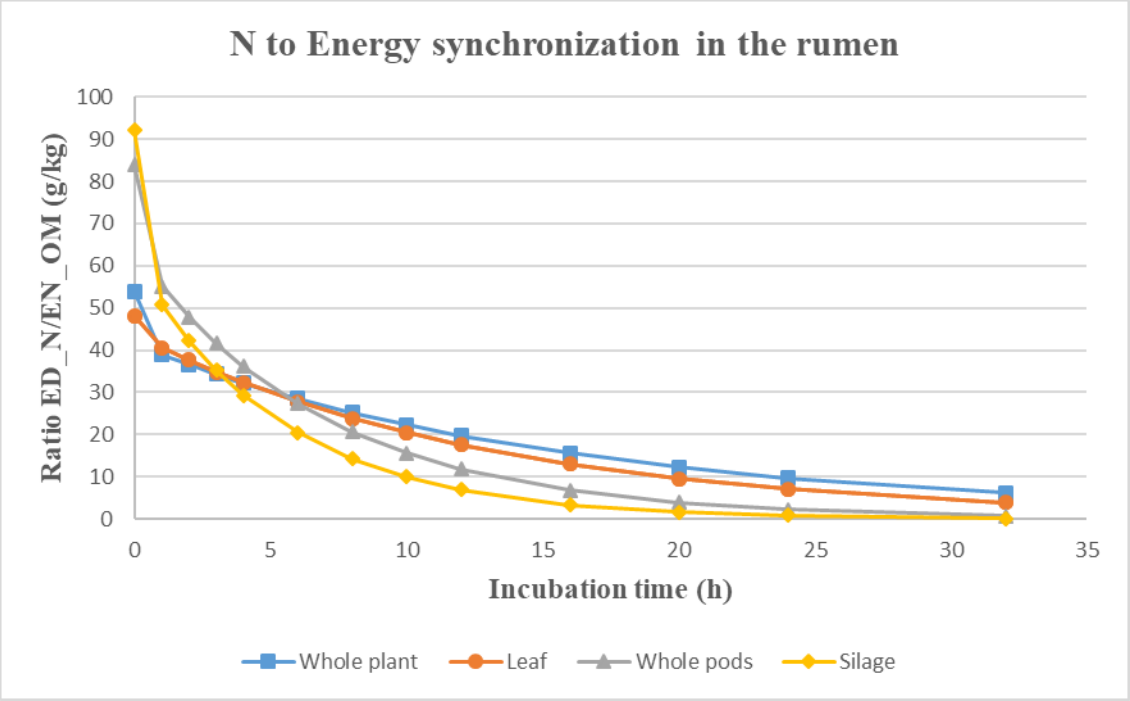


Figure 3.1. Hourly effective degradation ratios (ED_N/ED_OM) between available N and available OM of recently developed faba bean plants: Comparison among faba leaf, whole pods, whole plant, and whole plant silage.

3.6.6. Intestinal digestion of recently developed faba bean plants: comparison among faba leaf, stem, pods, whole plant and whole plant silage

Intestinal digestibility of rumen degradable protein was conducted with three-step *in vitro* method and the results are shown in Table 3.8. According to the results, WP had the highest estimated intestinal digestibility and it was numerically higher than WPL (87.24 % compared with 78.12 %) and WPL was numerically higher than silage (66.85%). Stem and leaf had the lowest intestinal digestibility.

Table 3.8. Intestinal digestion of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage.

	Faba bean partitions						
Item	WPL	Stem	Leaf	WP	Silage	SEM	*P* value
RUP Intestinal digestibility (%)							
IDRUP	78.12^{ab}	46.83^{c}	43.03^{c}	87.24^{a}	66.85^{b}	3.493	<0.001

Notes: $^{a-c}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; IDRUP, intestinal digestibility of rumen undegradable protein.

3.6.7. Modeling truly absorbable nutrient supply to dairy cows from recently developed faba bean plants: comparison among faba leaf, stem, pods, and whole plant and comparison among models (DVE/OEB vs NRC-2001)

The results of metabolizable protein supply and feed milk value of faba bean partitions and faba bean silage with DVE/OEB and NRC-2001 systems are showed in Tables 3.9 and 3.10 respectively. The supply of metabolizable protein to small intestine in the ruminant has three origins, microbial protein, undegradable feed protein and endogenous protein, while the first two fractions contribute to most of it. To maintain the normal function of rumen and to maximize the feed efficiency, microbial protein synthesize should be maximized, however, energy is not always synchronize with microbes requirement (Firkins 1996). To reflect the different energy supply conditions, microbial protein synthesis with and without sufficient energy were both considered in two systems.

In both systems, WP was predicted to have the highest microbial protein (MCP) production based on energy, followed by WPL and silage, while stem was the lowest ($P < 0.001$). In addition, two systems gave similar prediction about the microbial protein synthesized from rumen degradable protein, which was highest in WPL and lowest in stem.

As for endogenous protein, DVE/OEB system calculates it considering the digestibility of organic matter and regards it as a loss of total metabolizable protein, while NRC-2001 system has the prediction based barely on its DM content and considers it as a supplement of metabolizable protein (Theodoridou and Yu 2013a). As a result, two systems showed totally different pattern in this fraction. In NRC-2001 model, endogenous protein supply contributed to very limited variance of the metabolizable protein, although the significant difference between different groups ($P < 0.001$). While in DVE/OEB system, considerable difference can be found, the highest and lowest

values of ENDP were 41.8 and 7.7 g/kg DM in stem and WP respectively ($P < 0.001$). However, as for feed origin metabolizable protein, two systems had the same prediction, from which WP, WPL and silage were significantly higher than stem and leaf ($P < 0.001$).

In regard with the metabolizable protein supply and feed milk value (FMV), trends were similar within both systems. In NRC-2001 system, WP, WPL and silage had the highest metabolizable protein and feed milk value, while stem had the lowest nutritive value ($P < 0.001$). In DVE/OEB system, WP was the highest for metabolizable protein and feed milk value, which was higher than values of alfafa (*Medicago sativa* L.) and timothy (*Phleum pratense* L.) in other studies. WPL was also found to be comparable with other types of forages (Yu et al. 2003; Lei et al. 2019). As for degraded protein balance (OEB), according to Lei et al. (2019), positive OEB value reflects lose of N from the rumen, while negative OEB value shows protein synthesis is compromised by lack of N source. As a result, in this study, WP, WPL, silage and leaf need to cooperate with rapid degradable energy source to maximize microbial protein synthesis.

Table 3.9. Predicted values of potential nutrient supply to dairy cattle of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage using the Dutch DVE/OEB system.

	Faba bean partitions						
Item (g/kg DM)	WPL	Stem	Leaf	WP	Silage	SEM	*P* value
Absorbable microbial protein synthesis in the rumen ($AMCP^{DVE}$)							
FOM	705^{b}	351^{d}	629^{c}	827^{a}	644^{bc}	14.6	<0.001
MCP_{FOM}	105^{b}	52^{d}	94^{c}	124^{a}	96^{bc}	2.2	<0.001
$MCP_{RDP}{}^{DVE}$	153^{c}	15^{d}	151^{c}	241^{a}	175^{b}	4.9	<0.001
$AMCP^{DVE}$	67^{b}	33^{d}	60^{d}	79^{a}	61^{bc}	1.4	<0.001
Endogenous protein in the small intestine (ENDP)							
ENDP	15^{c}	41^{a}	14^{c}	7^{d}	19^{b}	0.9	<0.001
Truly absorbable rumen-undegraded protein in small intestine ($ARUP^{DVE}$)							
RUP^{DVE}	34^{bc}	33^{cd}	40^{a}	30^{d}	37^{b}	0.7	<0.001
$ARUP^{DVE}$	26^{a}	14^{b}	17^{b}	26^{a}	24^{a}	1.2	<0.001
Total truly digested protein in small intestine (DVE value)							
DVE	78^{b}	7^{d}	62^{c}	97^{a}	66^{bc}	3.3	<0.001
Degraded protein balance (OEB value)							
DPB^{DVE}	47^{c}	-37^{d}	57^{bc}	117^{a}	79^{b}	4.9	<0.001
Feed Milk Value (kg milk/kg DM feed)							
FMV	1.60^{b}	0.15^{d}	1.28^{c}	1.99^{a}	1.35^{bc}	0.066	<0.001

Notes: $^{a\text{-}c}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; FOM, organic matter fermented in the rumen; MCP_{FOM}, microbial protein synthesized in the rumen based on available energy; $MCP_{RDP}{}^{DVE}$, microbial protein synthesized in the rumen based on rumen degraded feed crude protein; $AMCP^{DVE}$, truly absorbed rumen synthesized microbial protein in the small intestine; ENDP, endogenous protein losses in the digestive tract; $ARUP^{DVE}$, truly absorbed bypass feed protein in the small intestine; RUP^{DVE}, ruminally undegraded feed CP, calculated according the formula in DVE/OEB system; dRUP, intestinal digestibility of rumen undegraded crude protein, estimated according to Tamminga et al. (1994); DVE, truly absorbed protein in the small intestine contributed by (1) feed protein escaping rumen degradation (RUP^{DVE}), (2) microbial protein synthesized in the rumen (MCP_{FOM}), and (3) a correction for endogenous protein losses in the digestive tract (ENDP); DPB^{DVE}, reflects the difference between the potential microbial protein synthesis based on rumen degraded feed crude protein (CP) and that based on energy (rumen fermented OM) available for microbial fermentation in the rumen; FMV, feed milk value.

Table 3.10. Predicted values of potential nutrient supply to dairy cattle of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage using the NRC-2001 model.

	Faba bean partitions						
Item (g/kg DM)	WPL	Stem	Leaf	WP	Silage	SEM	*P* value
Absorbable microbial protein synthesis in the rumen ($AMCP^{NRC}$)							
MCP_{TDN}	83^{bc}	64^{d}	80^{c}	91^{a}	84^{b}	0.899	<0.001
$MCP_{RDP}{}^{NRC}$	133^{c}	16^{d}	133^{c}	208^{a}	152^{b}	4.135	<0.001
$AMCP^{NRC}$	53^{bc}	10^{d}	51^{c}	59^{a}	54^{b}	0.575	<0.001
Absorbable endogenous true protein in the small intestine (AECP)							
ECP	11.02^{c}	11.15^{b}	10.94^{d}	10.98^{cd}	11.31^{a}	0.016	<0.001
AECP	4.41^{c}	4.46^{b}	4.37^{d}	4.40^{cd}	4.52^{a}	0.006	<0.001
Truly absorbable rumen-undegraded protein in small intestine ($ARUP^{NRC}$)							
RUP^{NRC}	30^{bc}	29^{cd}	35.92^{a}	27^{d}	32^{b}	0.590	<0.001
$ARUP^{NRC}$	23^{a}	13^{b}	15.46^{b}	23^{a}	22^{a}	1.095	<0.001
Total metabolizable protein (MP)							
MP	81^{a}	29^{c}	71^{b}	87^{a}	80^{a}	1.423	<0.001
Degraded protein balance (DPB^{NRC})							
DPB^{NRC}	59^{c}	-56^{d}	62^{bc}	137^{a}	80^{b}	4.240	<0.001
Feed Milk Value (kg milk/kg DM feed)							
FMV	1.64^{a}	0.58^{c}	1.43^{b}	1.76^{a}	1.63^{a}	0.030	<0.001

Notes: $^{a-d}$Means with different letters in the same row are significantly different ($P < 0.05$). SEM, Standard Error of Mean. Multi-treatment comparisons using Tukey method. WPL, whole plant; WP, whole pods; MCP_{TDN}, microbial protein synthesized in the rumen based on available energy (discounted TDN); $MCP_{RDP}{}^{NRC}$, microbial protein synthesized in the rumen based on available protein calculated as 0.85 of rumen degraded protein; $AMCP^{NRC}$, truly absorbed rumen-synthesized microbial protein in the small intestine; ECP, rumen endogenous crude protein (CP); AECP, truly absorbed endogenous protein in the small intestine; RUP^{NRC}, ruminally undegraded feed CP, calculated according the formula in NRC-2001 dairy model; dRUP, intestinal digestibility of rumen undegraded crude protein, estimated according to Tamminga et al. (1994); $ARUP^{NRC}$, truly absorbed rumen-undegraded feed protein in the small intestine; MP, metabolizable protein (true protein that is digested post-ruminally and the component amino acid absorbed by the intestine) contributed by (1) ruminally undegraded feed CP, (2) ruminally synthesized microbial CP, and (3) endogenous CP; DPB^{NRC}, reflects the difference between the potential microbial protein synthesis based on ruminally degraded feed CP and that based on energy-TDN available for microbial fermentation in the rumen; FMV, feed milk value.

3.7. Chapter conclusions

Faba bean partitions and faba bean forage had significantly different chemical compositions and nutritional values. Faba bean whole plant after ensiling had higher CP and starch content. Stem of faba bean had the highest NDF, ADF and ADL content and the lowest CP, SCP, and starch content. Leaf of faba bean had the lowest NDF, ADF and ADL content and the highest sugar, EE and had comparable CP content with WPL; however, the proportion of faba bean leaf in whole plant was only 6.1 %, which constrains its application.

Energy value of faba bean samples basically followed the same trend, which was the highest for WP, secondary for silage and WPL, followed by leaf and then the lowest for stem. As for CNCPS fractions, faba bean silage compared with whole plant faba bean had numerically higher rumen degradable carbohydrate fractions (RDCHO) and higher rumen degradable crude protein (RDCP). WP had similar RDCHO with silage and WPL but had much higher (30%) RDCP. According to the rumen degradation kinetics, faba bean silage had mismatching N and energy supply with N quickly released during first few hours of degradation. The same was true for WPL.

Metabolizable protein supply and feed milk value were predicted to be slightly different with DVE/OEB and NRC-2001 system. In DVE/OEB system, WP had the highest metabolizable protein (MP) supply and feed milk value (FMV). WPL was numerically higher than silage and silage was numerically higher than leaf. However, in NRC-2001 system, WPL, WP and silage were predicted to have similar MP and FMV.

In terms of metabolizable protein supply, WP, WPL and faba silage were all found to be comparable or higher than commonly used forage source of alfalfa hay, and timothy, compared with the results of other studies (Yu et al. 2003a; Yari et al. 2012). In addition, the energy value of WP, WPL and faba silage were also found to be similar or higher than alfalfa hay and timothy and

corn silage (Yu et al. 2003b; Schroeder 2004). As a result, faba bean WP, WPL and silage are nutritionally comparable with other forage sources such as alfalfa and timothy and may be used as alternative forage source. However, according to the results of this study, unbalanced energy and protein supply was observed in rumen degradation. Therefore, feeding faba bean WP, WPL and silage may lead to unhealthy rumen function such as acidosis and bloat. Therefore, faba bean WPL, WP and faba silage may need to combine with other forage sources with enough physically effective fiber. Nevertheless, different feed resource will lead to unpredictable rumen microbiology reaction. Hence, combination of feed should be fed in realistic case in order to figure out whether it is applicable in feeding. This study only analyzed possibility of using faba bean as forage resource according to chemical composition and ruminal digestion. Animal trial to feed faba bean to dairy cattle should be conducted to verity the possibility.

The choice of forage attaches great importance to making most use of local resources. Considering the fluctuation of production and market of different forage sources, faba bean could provide farmer with more choice under different market and environmental condition. In addition, the N flexing ability of faba bean enables it to be used for crop rotation and afterwards possible to be used as forage source for livestock. In the province of Saskatchewan, the annual participation is usually lower than what required for optimal growth, while in northern Saskatchewan, the participation slightly higher than in the south and middle (Environment and Climate Change Canada 2011), therefore, faba bean is more suitable to be planted in the northern part of the province.

Considering the limitation of yield, intercropping may be considered to improve its yield. In the meantime, high readily digestible carbohydrate and protein fractions in whole plant faba bean could be problematic and thus needing to cooperate with other forage sources such as maize and

barley which have high starch content but low in CP. Intercropping is a common practice which provides numerous benefits such as improved yield and land efficiency and decreased disease and weeds. In addition, in this study, the supply of energy cannot match up with the supply of nitrogen in whole plant and whole plant silage, however, when faba bean is fed together with other crops which have high readily digestible carbohydrate content but lack in protein, high soluble carbohydrate fraction in these crop could cooperate with high readily digestible protein fraction in faba bean whole plant and thus acquiring better animal performance. Researches have been done to test the potential of intercrop faba bean with other crops and positive results were acquired. When faba bean was intercropped with maize in Sweden, decreased yield was observed compared with sole maize, however, slighter higher forage protein content was seen (mean increase 10-15 g/kg DM) (Stoltz et al. 2013). When wheat was interplanted with faba bean decreased DM yield compared with sole wheat was observed, however, enhanced CP and NDF and WSC was observed for faba bean and wheat respectively when interplanted (Ghanbari-Bonjar and Lee 2002). Moreover, when faba bean was intercropped with rye (especially in 50:50 seeding ratio), higher forage and CP yield per hectare was observed than monocrops (Lithourgidis and Dordas 2010). However, climate and soil conditions should be considered as well as the effect of different variety when applied to western Canada. In conclusion, faba bean WP, WPL and faba silage may be used as alternative forage source nutritionally and economically with attention to corporate with other forage sources. Further study could also be done about intercropping faba bean with other forage resources in western Canada, and the nutritional characteristics of ensiling faba bean with other crops.

Overall, following conclusions could be made: (1) faba bean partitions proportion highly variable; (2) Nutritional composition of faba bean partitions highly variable with WP had the highest

nutritional value followed by WPL and faba silage and (3) whole plant, whole pods and faba silage could be used as potential feed ingredient for dairy cows.

4. MOLECULAR STRUCTURE SPECTRAL PROFILES OF RECENTLY DEVELOPED FABA BEAN PLANTS, REVEALED BY VIBRATIONAL MOLECULAR SPECTROSCOPY: COMPARISON AMONG LEAF, STEM, WHOLE PODS, WHOLE PLANT AND WHOLE PLANT FABA SILAGE BEFORE AND AFTER RUMEN DIGESTION.

4.1. Abstract

The aim of this study was to explore the function of ruminal digestion to the change of molecular spectral features of different faba bean samples using vibrational molecular spectroscopy with both univariate and multivariate analyses. Samples after grounded through 0.12 mm sieve were performed with spectral analyses using a JASCO FT/IR–4200 spectroscope (JASCO Corp., Tokyo, Japan). Samples were scanned within mid-infrared region (ca. 4000-700 cm^{-1}) at 4 cm^{-1} resolution. OMNIC 7.4 software (Spectra Tech, Madison, WI) was used for measuring peak height and peak area in univariate analysis. Unscrambler X software (v.10.3, Camo Software AS) was used for multivariate analysis of principal component analysis (PCA) in this study.

According to the results, for univariate analysis of protein related spectral profiles, faba bean samples were interacted with incubation time for most of the spectral profiles; different patterns were observed for different faba bean samples in different spectral parameters. The same is true for carbohydrate related spectral parameters. For protein related spectral parameters of peak height of beta sheet, amide I, peak area of amide II and area ratio of amide I to amide II, spectral intensity was decreased with increasing time of incubation. However, for carbohydrate related parameters of total carbohydrate 1^{st} peak height and cellulosic compound peak height and peak area, spectral intensity was increased after incubated in the rumen. For multivariate analysis, spectra of original faba bean samples could be separated while 12 h and 24 h incubation residue samples could not

be separated from each other in carbohydrate and protein related regions. Overall, spectral analysis provides us with additional molecular information that traditional chemical analyses cannot achieved. Further study is needed to figure out the biological meaning of the spectral change during ruminal digestion.

4.2. Introduction

The intrinsic molecular structure of feed is indispensable in acquiring the full feeding information of the feed. According to published papers (Xin et al. 2014; Xin and Yu 2014), carbohydrate related spectral features are correlated to nutrient values and may be used to predict nutritional supply to animal. In addition, feeding value and protein digestive behavior are also influenced by protein structural features, which is because protein secondary structure is highly associated with the specific susceptibility to the enzymatic hydrolysis (Yu 2005b, 2007). According to Stuart (2015), the characteristics of a group frequency are mainly reflected by its region in spectrum; within the mid-infrared region, it can be further divided into X-H stretching region (ca. 4000 to 2500 cm^{-1}), triple bond region (ca. 2500 to 2000 cm^{-1}), double bond region (ca. 2000 to 1500 cm^{-1}) and the fingerprint region (ca. 1500 to 600 cm^{-1}) (Stuart 2015). The vibrations in X-H stretching region are generally because of the stretch between the hydrogen atom and atoms like carbohydrate, oxygen and nitrogen, while triple bond stretching absorptions are due to C≡C and C≡N and sometimes X-H stretching, where X is atom with bigger atomic mass like silicon and phosphorus. The vibration in double bond region is generally due to C=C, C=O and C=N stretching with the C=C stretching one of the most noticeable absorption in an infrared spectrum. By associating the unique absorption of infrared radiation to its chemical bonds, the chemical composition and structural information can be acquired.

Nevertheless, the harsh chemical reactions during traditional chemical analytical method destroy feed molecular structure, while Fourier transform infrared spectroscopy (FTIR) can detect the molecular structure with intact samples (Yu et al. 2004b). In addition, FTIR spectroscopy is a rapid analytical technique that has been demonstrated to be able to detect the molecular chemistry in different biological components (Yu 2005a, 2007). As a result, FTIR is utilized to reveal the association between internal molecular structure and nutritional and digestive characteristics of feeds, as well as the possible alteration of structure during processing (Rodríguez-Espinosa et al. 2019).

Although FTIR has been utilized to assist feed evaluation in many studies, study about the influence of microbial degradation to molecular structure is limited. Studies conducted by Xin and Yu (2013b, 2013c, 2014), compared the alteration of spectral profiles of canola and *Brassica carinata* during microbial digestion and found out the change of molecular structure of carinate and canola meal during rumen incubation and its association with basic nutrient compositions. However, only chemical profiles were used to correlate with structural change, which cannot reflect the utilization and digestion condition of the feed.

In mid-infrared region, spectral absorption of the specific chemical bond can appear in different wavenumber in different compounds. For feed samples which include sophisticated chemical bonds, the spectral features of grounded feed samples basically have spectral absorption of total carbohydrate region (ca. 938-1186 cm^{-1}), structural carbohydrate region (ca. 1186-1486 cm^{-1}), cellulosic compound region (ca. 1186-1289 cm^{-1}), amide region (ca. 1486-1715 cm^{-1}) and lipid related region (ca. 2844-3005 cm^{-1}). Within each region several peaks are observed, which is the comprehensive results of complicated chemical bonds and interaction of different compounds.

4.3. Study objectives

The objectives of this study was to compare molecular spectral features among leaf, stem, whole pods, whole plant and whole plant faba bean silage and determine the change of spectral features of different faba bean samples during 12 and 24 h rumen degradation using both univariate molecular spectral analysis and multi-variate molecular spectral analysis.

4.4. Study hypotheses

The study hypothesis was that spectral features could differ among different faba bean samples and the function of ruminal digestion to the change of spectral features could be reflected in both univariate and multivariate analyses.

4.5. Materials and Methods

4.5.1. Sample preparation

All sample for spectral analysis were grounded through 0.12mm sieve. Fourier transform Infrared (FTIR) spectra of faba bean samples before and after 12 and 24 h incubations were collected with JASCO FT/IR-4200 with ATR (JASCO Corp., Tokyo, Japan). Each sample was collected for five spectra at mid-IR range (ca. 4000-700 cm^{-1}) with 128 scans and at 4 cm^{-1} resolution. Overall, 15 faba bean samples of both original and 12 and 24 h incubation residues were collected for spectra (n=15×3). Background spectrum was collected with 256 scans before each sample to minimize background noise. The spectral parameters include peak height and area, which were measured with OMNIC 7.4 software (Spectra Tech, Madison, WI, USA); area and height ratios were calculated based on relevant spectral profiles. According to published paper (Yu 2007), protein related spectral parameters were identified, which are amide region (ca. 1730-1480 cm^{-1}), amide I region (ca. 1713-1558 cm^{-1}) and amide II region (ca. 1558-1485 cm^{-1}). Within amide I region, protein secondary structural features of α-helix (around ca. 1644 cm^{-1}) and β-sheet (around ca.

1630 cm^{-1}) were distinguished, two pre-possessing (secondary derivative and automatic smooth) were processed to identify these two regions. Within the mid-infrared region, in addition to amide I, amide II region, there is also a region correlated with protein secondary structure namely amide III region (ca. 1200-1400 cm^{-1}), however spectral features in amide II and amide III region have strong interaction with lignin (Salas et al. 2014), therefore, for plant-based samples amide II and amide III regions are excluded for protein secondary structure measuring. In addition, carbohydrate related spectral regions were also identified, which include total carbohydrate region (ca. 938-1186 cm^{-1}), cellulosic compound region (ca. 1186-1289 cm^{-1}) and structural carbohydrate region (ca. 1186-1486 cm^{-1}). Multivariate approach principle component analysis (PCA) was conducted in this study at amide region, structural carbohydrate region and total carbohydrate region. Unscrambler X software (v.10.3, Camo Software AS) was used for PCA analysis in this study.

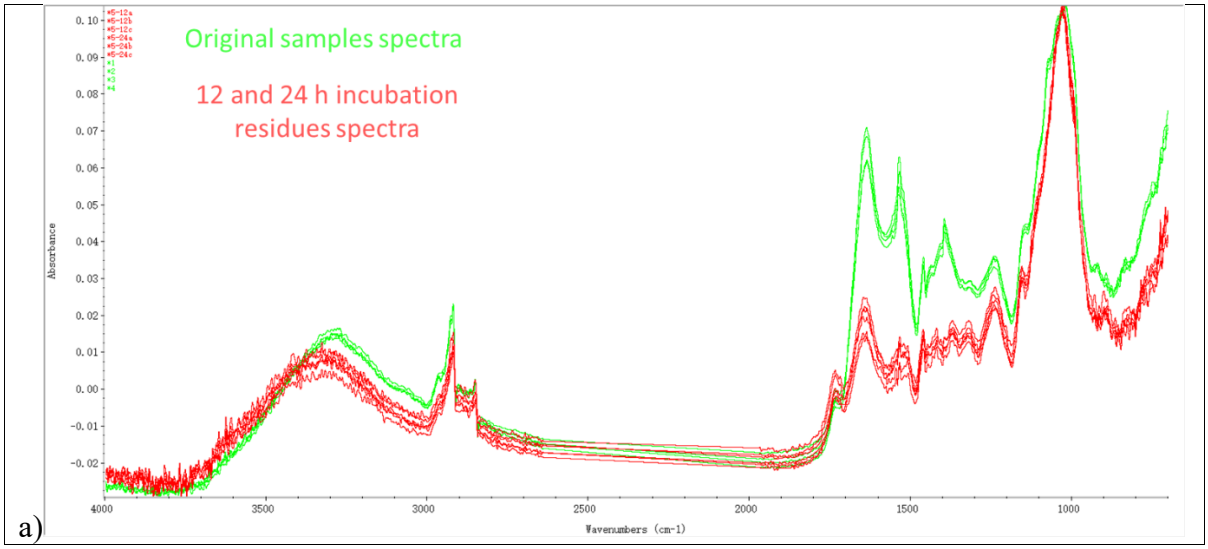
4.5.2. Statistical analyses

Completely random design was used for spectral analyses, and the model was: $Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + Error_{ij}$, Y_{ij} was the observation of the dependent variable ij; μ was the population mean for the variable; $\alpha_i$was the effect of different faba bean partitions and faba forage (i = 1-5); $\beta_j$was the effect of incubation time (j = 1-3); $(\alpha\beta)_{ij}$ was the interaction between α_i and β_j; $Error_{ij}$ was the random error associated with observation ij. Normality of the residual data was checked using Procedure Univariate of SAS with Shapiro-Wilk method. Multi-comparisons were performed with Tukey-Kramer method. For all statistical analysis, significance was declared at $P < 0.05$ and trends at $0.05 \leq P \leq 0.10$.

4.6. Results and Discussion

4.6.1. Univariate molecular spectral analysis of protein and carbohydrate profiles before and after rumen digestion

The whole spectral profiles of each faba bean sample were shown in Figures 4.1, 4.2 and 4.3. According to the results, greater change of spectral feature during rumen incubation were observed at amide region (ca. 1486-1715 cm^{-1}) and structural carbohydrate region (ca. 1186-1486 cm^{-1}) than in total carbohydrate region (ca. 938-1186 cm^{-1}) and in total carbohydrate region, minimal change was observed during the incubation, especially in the peak around ca. 1024 cm^{-1}. In addition, in spectra of whole pods samples, a gradual decrease of spectral intensity during increase of incubation time was seen in amide region which was not seen in other samples. While in spectral of stem, there was minimal change in the spectral intensity.



a)

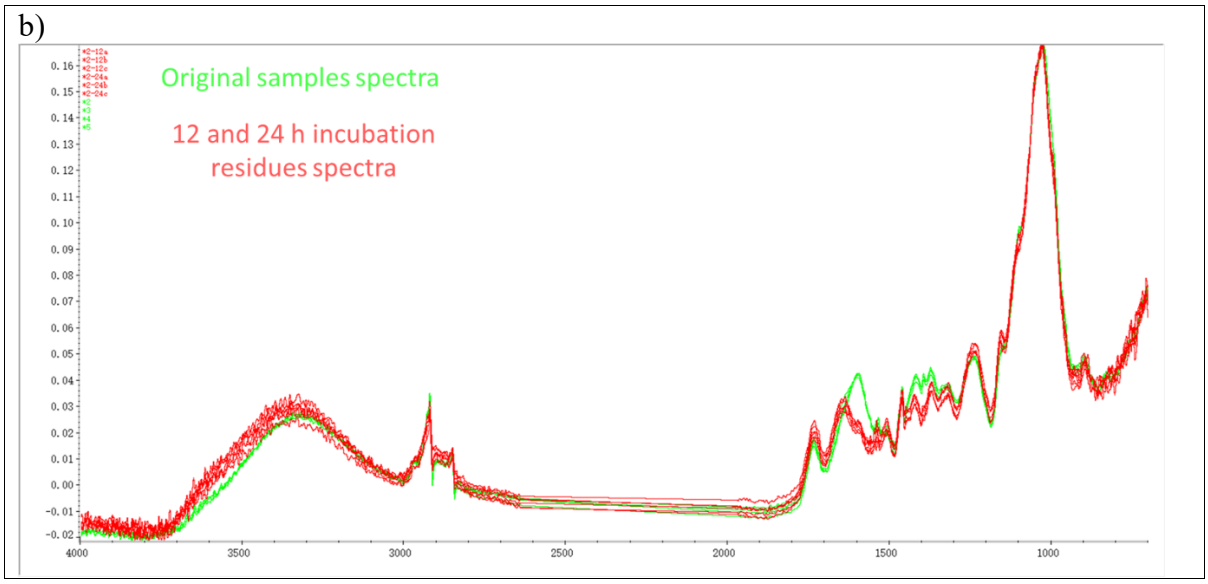


Figure 4.1. Spectra of original and 12 and 24 incubation residue samples within whole mid-infrared region of a) whole plant faba bean and b) stem of faba bean

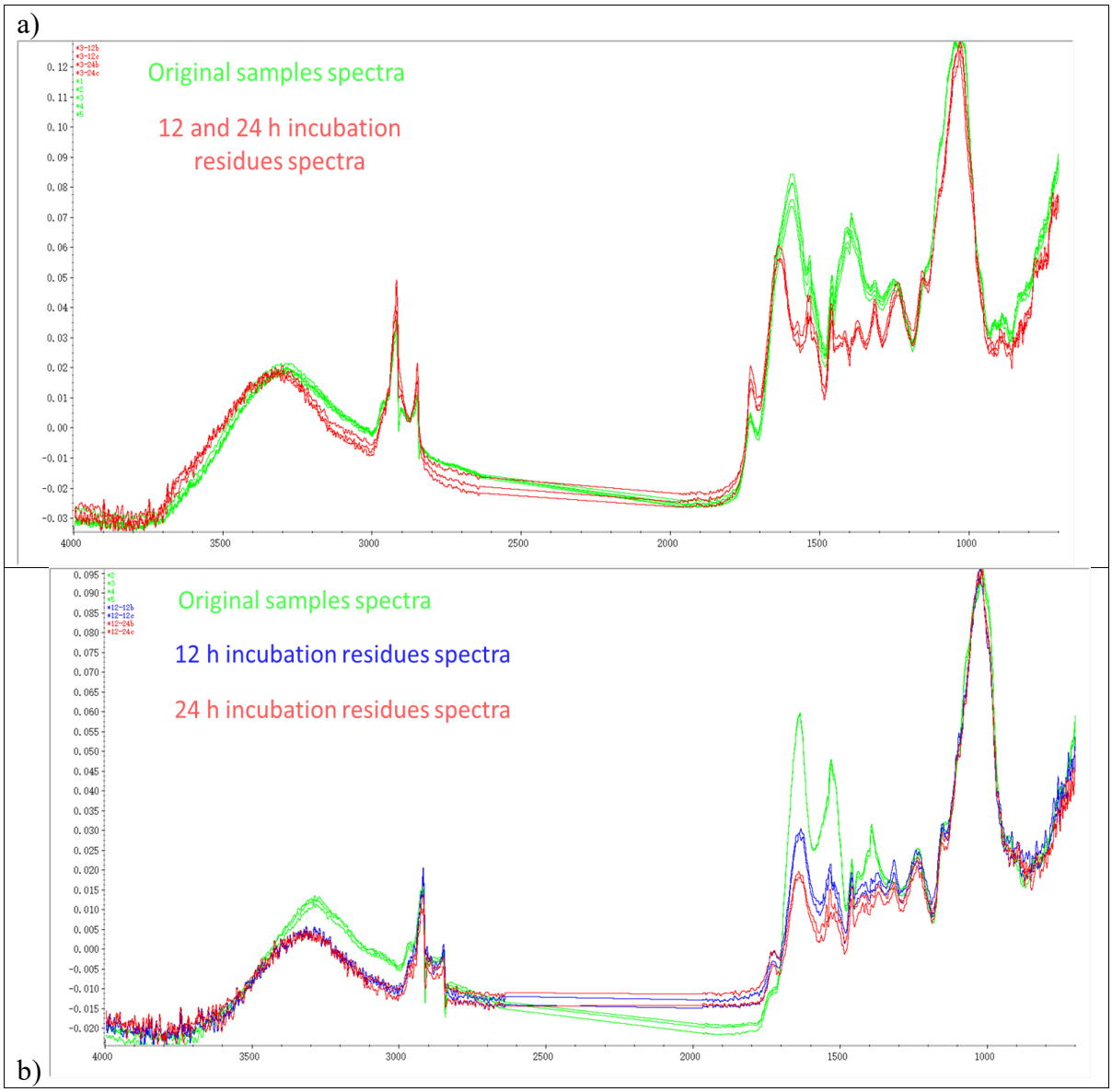


Figure 4.2. Spectra of original and 12 and 24 incubation residue samples within whole mid-infrared region of a) leaf of faba bean and b) whole pods of faba bean

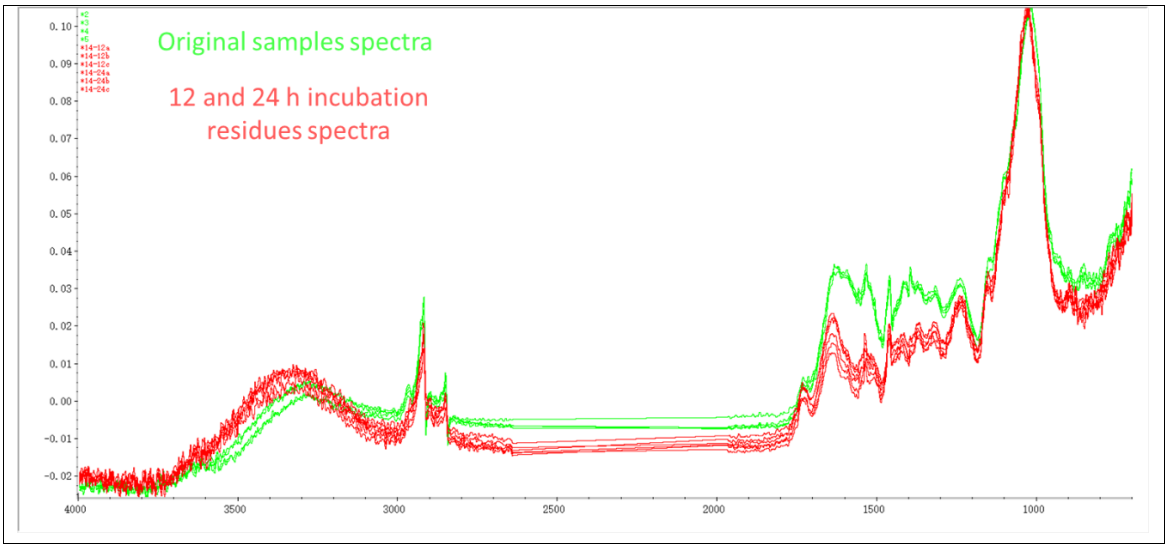


Figure 4.3. Spectra of original and 12 and 24 incubation residue samples within whole mid-infrared region of faba bean silage

Protein related spectral profiles analyzed by univariate analysis were showed in Table 4.1 According to the results, there were significant interaction between faba bean partitions and faba silage and incubation time in spectral parameters of amide II peak height, α-helix peak height, amide I area, amide I to amide II peak height ratio and α-helix to β-sheet peak height ratio. Especially in amide I to amide II peak height ratio and α-helix to β-sheet peak height ratio, there were strongly significant interaction effect ($P < 0.001$) and the results are illustrated in Figure 4.4 According to Figure 4.1 (A), the AIH/AIIH was increasing in all treatments after first 12 h of incubation, while in stem a much higher increase was observed; in the second 12 h, dramatic increase was seen in stem, while in other treatments the values had minimal change or slightly increase. As for α-helix to β-sheet ratio, an increase was found in all treatments except WP, and stem had the highest increase; WP instead, decreased in first 12 h of incubation. Between 12 and 24 h incubation, only silage maintained its increase, when other treatments had litter change. The study by Xin and Yu (2013a) demonstrated the effect of microbial digestion to change of the molecular structural in rapeseed meal and found the continuous change of protein molecular

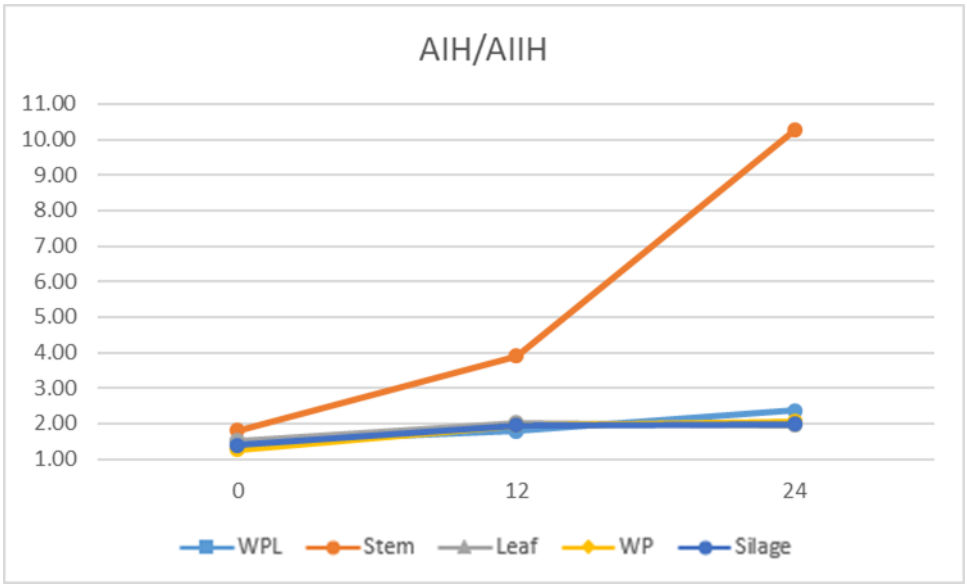
structure during incubation, which were partially consistent with the results in this study. In the study of Xin and Yu (2013b), the interaction between Carinate and Canola meals and incubation time was not significant in all protein related spectral parameters while in this study, the interaction is significant in some of the spectral profiles especially in α-helix to β-sheet ratio and amide I to amide II peak height ratio. The different results may because of the relatively similar chemical compositions and spectral profiles between Carinate and Canola meals, while in this study chemical compositions, spectral profiles and protein metabolism and digestion characteristics were all significantly different among faba bean partition samples.

However, in spectral parameters of β-sheet peak height, amide I peak height, amide peak area, amide II area, they were significantly altered by increase of incubation time and the alteration were different among faba bean samples. Specifically, the highest value could be found in WP and leaf, followed by WPL and silage, with the lowest value in stem, which was consistent with its protein content and digestive values. The spectral ratios between amide I and amide II show another pattern, where stem was significantly higher than other treatments ($P < 0.001$). As for the effect of incubation time for these spectral parameters, after rumen incubation the spectral profiles were significantly decreased ($P < 0.001$), which was consistent with the results of Xin and Yu (2013a). However, in this study the difference between 12 and 24 h incubation samples were not significant.

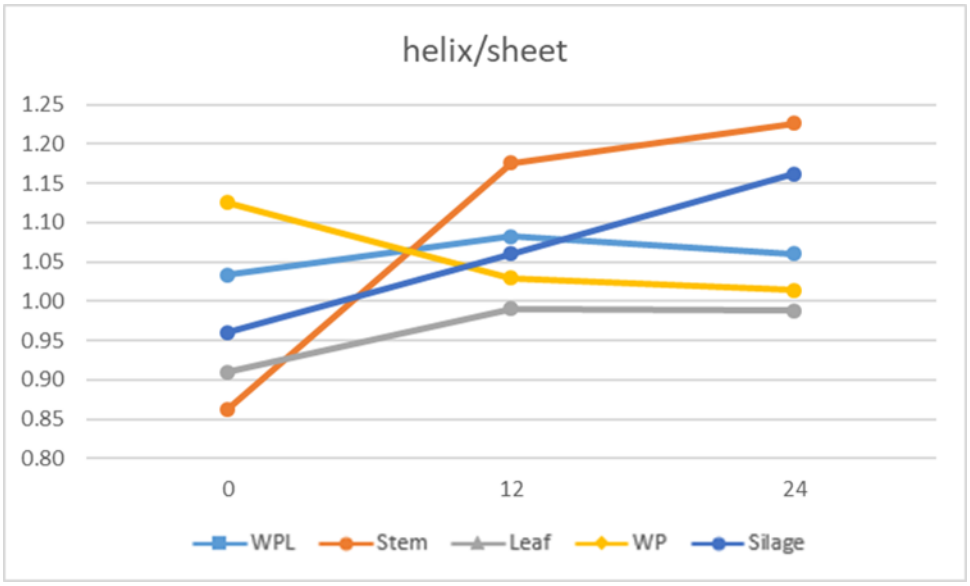
Table 4.1. Protein related spectral profiles of recently developed faba bean plants: Comparison among faba leaf, stem, whole pods, whole plant and silage before and after 12 and 24 h rumen incubation.

	Treatment						Incubation time				*P* value		
Item	WPL	Stem	Leaf	WP	Silage	SEM	0	12	24	SEM	Trt	Inc.t	Trt*Inc.t
AIIH	0.148	0.046	0.164	0.199	0.107	0.0127	0.209	0.103	0.0867	0.0115	<0.001	<0.001	0.012
Beta	0.232^{b}	0.099^{d}	0.287^{ab}	0.314^{a}	0.166^{c}	0.314	0.294^{A}	0.193^{B}	0.171^{B}	0.0136	<0.001	<0.001	0.133
AIH	0.242^{b}	0.105^{d}	0.289^{ab}	0.319^{a}	0.174^{c}	0.0147	0.295^{A}	0.202^{B}	0.179^{B}	0.0132	<0.001	<0.001	0.196
Helix	0.243	0.102	0.274	0.333	0.173	0.0172	0.296	0.201	0.178	0.0155	<0.001	<0.001	0.005
AA	27.707^{bc}	11.536^{d}	34.592^{ab}	38.027^{a}	19.993^{c}	1.999	39.241^{A}	21.337^{B}	18.536^{B}	1.803	<0.001	<0.001	0.067
AIIA	6.984^{bc}	2.498^{d}	8.089^{ab}	9.808^{a}	5.122^{c}	0.651	9.286^{A}	5.473^{B}	4.741^{B}	0.589	<0.001	<0.001	0.072
AIA	20.722	9.038	26.504	28.220	14.871	1.374	29.955	15.863	13.796	1.238	<0.001	<0.001	0.044
AIA/AIIA	3.076^{b}	3.66^{a}	3.224^{ab}	2.910^{b}	2.925^{b}	0.128	3.442^{A}	3.086^{B}	2.949^{B}	0.115	<0.001	<0.001	0.074
AIH/AIIH	1.877	5.321	1.830	1.761	1.781	0.313	1.493	2.324	3.725	0.282	<0.001	<0.001	<0.001
helix/sheet	1.059	1.088	0.963	1.056	1.061	0.0174	0.978	1.068	1.090	0.0158	<0.001	<0.001	<0.001

Notes: Trt, treatment; Inc.t, incubation time. Letters in lower case represent significance between treatments, letters in upper case mean significance between incubation time. SEM, standard error of the mean. WPL, whole plant; WP, whole pods. AIH, peak height of amide I; AIIH, peak height of amide II; helix, peak height of α-helix; sheet, peak height of β-sheet; AA, area of amide region; AIA, area of amide I; AIIA, area of amide II; AIH/AIIH, peak height ratio of amide I and amide II; AIA/AIIA, peak area ratio of amide I and amide II; helix/sheet, peak height ratio of α-helix and β-sheet.



(A)



(B)

Figure 4.4. Interaction between incubation time and whole plant (WPL), stem, leaf, whole pods (WP) of faba bean and whole plant faba silage in regard with protein related molecular spectral profiles of (A) AIH/AIIH (amide I to amide II peak height ratio) and (B) helix/sheet (α-helix to β-sheet peak height ratio).

The results of carbohydrate related spectral profiles of faba bean partitions and faba bean forage are showed in Tables 4.2 and 4.3. Based on the results, faba bean samples were significantly interacted with incubation time for all the spectral profiles except for spectral features of total carbohydrate 1st peak height and peak height and peak area of cellulosic compounds. Similar study was conducted by Xin and Yu (2013c), which compared effect of ruminal digestion to carbohydrate related spectral profiles in Canola meal and Carinate meal, and the interaction between incubation time to feed types can also be found. In this study, it is interesting to find out in spectral parameters of total carbohydrate 1st peak height (wavenumber ca. 1024 cm^{-1}) and peak height and peak area of cellulosic compounds, spectral intensity was firstly increased after incubated in the rumen for 12 h and then had minimal change or slight increase when incubation time came to 24 h. According to reference, the peak ca. 1024 cm^{-1} is assigned to C-O stretching in cellulose and lignin (Traoré et al. 2016). In the study of Xin and Yu (2013b), feed samples were interacted with incubation time in all spectral parameters except for cellulosic compound peak height and peak area which is partly consistent with this study. In addition, in the research of Xin and Yu (2013c), the spectral intensity of samples after incubation were much lower than original samples spectral intensity which was not observed in this study. The inconsistent results between two studies demonstrates the complicated effect of microbial digestion to the molecular spectral profiles which may have relationship with the inherent chemical and structural difference between samples.

Table 4.2. Carbohydrate related spectral profiles (peak height) of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage before and after 12 and 24 h rumen incubation.

Carbohydrate related spectral profiles									
Item	Inc.t	TC1	TC2	TC3	TC4	CEC_H	STC1	STC2	STC3
WPL	0h	0.603	0.460^{b}	0.316^{bc}	0.161^{ab}	0.099	0.084^{def}	0.129^{bc}	0.172^{b}
	12h	0.644	0.437^{b}	0.311^{cd}	0.146^{abc}	0.107	0.091^{cdef}	0.1^{e}	0.079^{d}
	24h	0.647	0.433^{b}	0.305^{cde}	0.135^{bc}	0.111	0.085^{def}	0.097^{e}	0.066^{d}
Stem	0h	0.618	0.445^{b}	0.344^{ab}	0.140^{bc}	0.112	0.097^{abcde}	0.136^{b}	0.134^{c}
	12h	0.667	0.444^{b}	0.306^{cde}	0.140^{bc}	0.12	0.084^{def}	0.101^{e}	0.058^{d}
	24h	0.656	0.451^{b}	0.310^{cd}	0.138^{bc}	0.121	0.077^{ef}	0.097^{e}	0.053^{d}
Leaf	0h	0.563	0.513^{a}	0.373^{a}	0.142^{bc}	0.072	0.120^{a}	0.205^{a}	0.258^{a}
	12h	0.611	0.443^{b}	0.314^{bcd}	0.132^{bc}	0.096	0.094^{bcde}	0.1^{e}	0.071^{d}
	24h	0.584	0.422^{b}	0.304^{cde}	0.123^{c}	0.100	0.103^{abcd}	0.098^{e}	0.064^{d}
WP	0h	0.578	0.445^{b}	0.277^{e}	0.175^{a}	0.099	0.068^{f}	0.104^{de}	0.173^{b}
	12h	0.639	0.457^{b}	0.323^{bc}	0.146^{abc}	0.114	0.112^{abc}	0.11^{de}	0.079^{d}
	24h	0.638	0.449^{b}	0.315^{bc}	0.142^{bc}	0.113	0.116^{ab}	0.109^{de}	0.074^{d}
Silage	0h	0.567	0.364^{c}	0.284^{de}	0.135^{bc}	0.085	0.100^{abcde}	0.117^{cd}	0.140^{c}
	12h	0.644	0.424^{b}	0.308^{cde}	0.151^{abc}	0.107	0.093^{bcde}	0.102^{e}	0.077^{d}
	24h	0.645	0.433^{b}	0.307^{cde}	0.136^{bc}	0.114	0.081^{def}	0.099^{e}	0.066^{d}
	SEM	0.0132	0.0084	0.006	0.006	0.0034	0.0047	0.003	0.0059
P value									
Trt		<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
Inc.t		<0.001	0.367	0.030	0.001	<0.001	0.727	<0.001	<0.001
Trt*Inc.t		0.604	<0.001	<0.001	0.036	0.054	<0.001	<0.001	<0.001

Notes: WPL, whole plant; WP, whole pods; Trt, treatment; Inc.t, incubation time. SEM, standard error of the mean. TC1, total carbohydrate first peak height; TC2, total carbohydrate second peak height; TC3, total carbohydrate third peak height; TC4, total carbohydrate fourth peak height; CEC_H, cellulosic compound region peak height; STC1, structural carbohydrate first peak height; STC2, structural carbohydrate second peak height; STC3, structural carbohydrate third peak height.

Table 4.3. Carbohydrate related spectral profiles (peak area and ratio) of recently developed faba bean plants: comparison among faba leaf, stem, whole pods, whole plant and whole plant silage before and after 12 and 24 h rumen incubation.

Carbohydrate related spectral profiles							
Item	Inc.t	TC	CEC	STC	STC/ CEC	STC/ TC	TC/ CEC
WPL	0h	76.945^{ab}	5.182	27.361^{bc}	5.323^{bc}	2.818^{de}	14.888^{bc}
	12h	75.318^{abc}	5.455	21.989^{fgh}	4.034^{defgh}	3.425^{abc}	13.809^{bcd}
	24h	74.410^{abc}	5.837	21.059^{h}	3.621^{fgh}	3.541^{a}	12.763^{cde}
Stem	0h	79.228^{a}	5.991	27.811^{b}	4.646^{cde}	2.850^{de}	13.227^{bcde}
	12h	76.316^{ab}	6.608	21.404^{gh}	3.253^{gh}	3.565^{a}	11.590^{de}
	24h	75.771^{ab}	6.651	21.069^{h}	3.167^{h}	3.596^{a}	11.391^{e}
Leaf	0h	76.367^{ab}	3.914	37.303^{a}	9.531^{a}	2.048^{f}	19.510^{a}
	12h	71.066^{bcd}	4.815	21.246^{h}	4.417^{cdef}	3.345^{abc}	14.775^{bc}
	24h	69.089^{cd}	5.225	21.381^{h}	4.101^{defgh}	3.231^{abcd}	13.249^{bcde}
WP	0h	73.328^{abcd}	4.930	23.944^{defg}	4.903^{bcd}	3.067^{bcde}	14.952^{bc}
	12h	74.741^{abc}	5.885	24.565^{de}	4.194^{defg}	3.048^{cde}	12.816^{cde}
	24h	74.367^{abc}	5.856	24.165^{def}	4.132^{defg}	3.088^{bcde}	12.775^{cde}
Silage	0h	67.709^{d}	4.419	25.210^{cd}	5.714^{b}	2.686^{e}	15.352^{b}
	12h	74.555^{abc}	5.617	22.141^{efgh}	3.944^{efgh}	3.367^{abc}	13.277^{bcde}
	24h	73.968^{abcd}	6.038	21.321^{h}	3.531^{fgh}	3.472^{ab}	12.259^{de}
	SEM	1.2486	0.2129	0.4911	0.1817	0.0805	0.4393
				P value			
Trt		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Inc.t		0.307	<0.001	<0.001	<0.001	<0.001	<0.001
Trt*Inc.t		0.001	0.284	<0.001	<0.001	<0.001	0.001

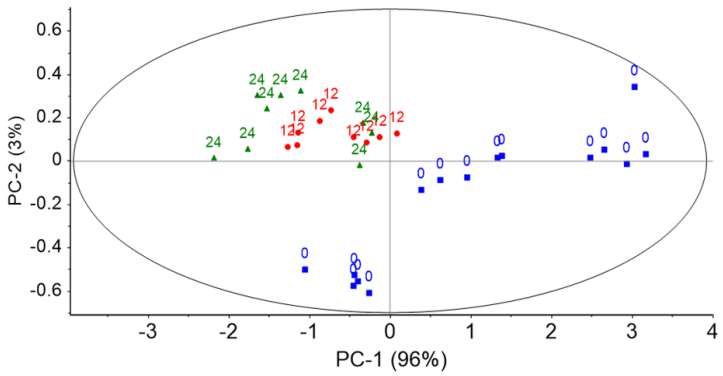
Notes: WPL, whole plant; WP, whole pods; Trt, treatment; Inc.t, incubation time. SEM, standard error of the mean. TC, total carbohydrate peak area; CEC, cellulosic compounds peak area; STC; structural carbohydrate peak area; STC/CEC, peak area ratio of STC to CEC; STC/TC, peak area ratio of STC to TC; TC/CEC, peak area ratio of TC to CEC.

4.6.2. Multivariate molecular spectral analysis for FTIR spectra: comparison among 0, 12 and 24 h digestion residues

Univariate analysis sometimes cannot differentiate the samples with selected peak height and area as only limited information contained in spectrum is used, in the meantime, multivariate analysis utilizing all information contained in the spectrum, overcomes the shortage of univariate analysis. In this study, to further prove the effect of incubation time to the molecular structural of faba bean partitions, PCA was used in the study. The results of multivariate analysis of protein related amide region was showed in Figure 4.2. Carbohydrate related spectral regions of structural carbohydrate and total carbohydrate regions were chose to perform for PCA analysis and the results are shown in Figures 4.3 and 4.4.

According to the results, faba bean samples before incubation were clearly separated with incubation residue samples, which is consistent with univariate results and reprove the statement that microbial digestion has a significant effect to feed molecular structure. As a result, PCA analysis can be used in future study to differentiate the subtle difference between different incubation time point residue samples, when univariate analysis fails to achieve. In this study, the spectra without any parameterization were used for multivariate analysis. Sometimes spectra with pre-processing such as secondary derivative and Fourier self-deconvolution, which help to reduce noise in physical variations and chemical interference and to separate overlapped peaks, were also used to develop regression model (Shi and Yu 2017). However, in this study due to the limitation of sample size, multi-linear regression was used instead of partial least square regression. In addition, mixture samples were used for spectral analysis, and interaction between protein, lipid and polysaccharides lead to complicated spectra and pre-processing samples may not be separated with PCA analysis. Therefore, only unprocessed samples were used for multivariate analysis.

The digestion of feed protein in the rumen involves with combination of various enzymes under specific order as feed protein, polysaccharides and even iron molecules are interacted with each other and form three dimension matrix structure which inhibit enzyme access (Wang and McAllister 2002). As a result, the cleave of bonds during ruminal digestion to allow proteolytic enzyme access is important for protein utilization and may correlated with its spectral profiles change. According to Yu (2004c), the low protein digestibility of feather protein is associated with its secondary structure as feather protein has a very high β-sheet ratio compared with other grains. In addition, other spectral profiles are also found to closely related to the nutritional profiles of feed protein, while the relationships differ between different feed varieties and processing methods (Li et al. 2016; Lei et al. 2019). In this study, the complicated relationship between incubation time and faba bean samples among various spectral profiles may demonstrate the importance of structural profiles to the digestion of protein during microbial digestion. Correlation study was further determined to prove the relationship between faba bean samples protein digestibility and spectral change.



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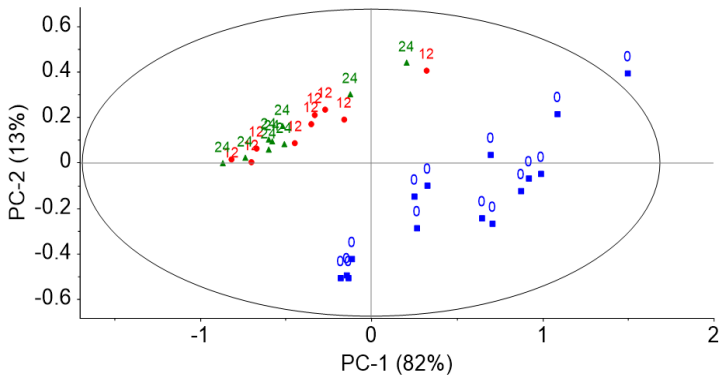

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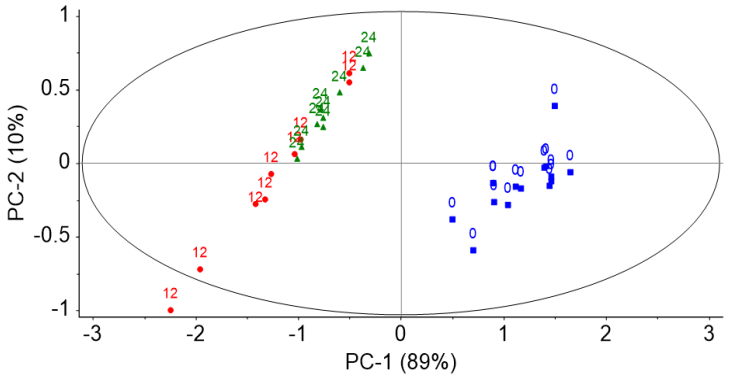

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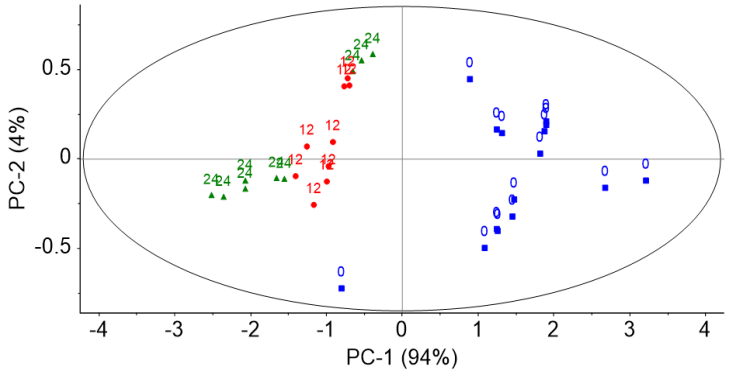

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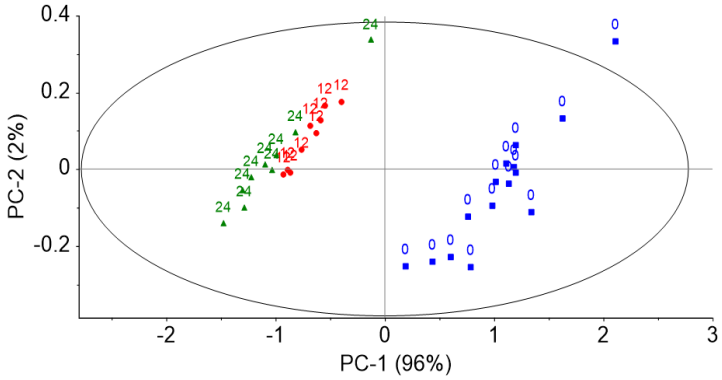
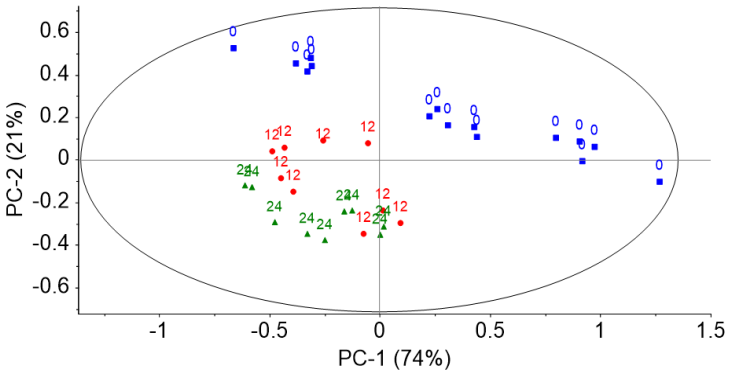


e)

Figure 4.2. PCA analysis of faba bean partitions and faba silage in amide region (ca. 1480-1730 cm^{-1}) of (a) whole plant, (b) stem, (c) leaf, (d) whole pods and (e) whole plant silage before rumen incubation (0 h) and after 12 and 24 h of incubation. Notes: 0, 12, 24 represent original samples, 12 h incubation residue samples and 24 h incubation residue samples.



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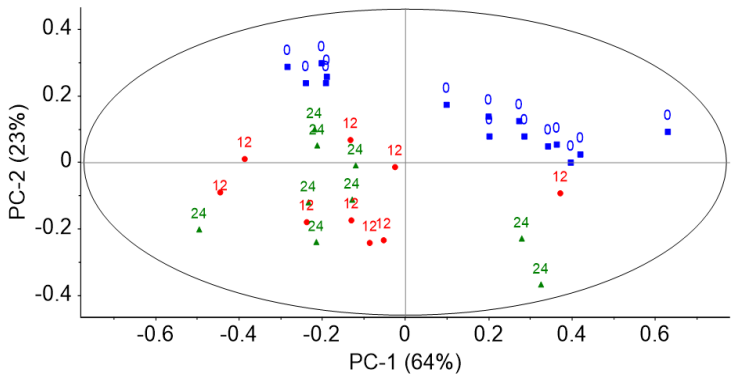

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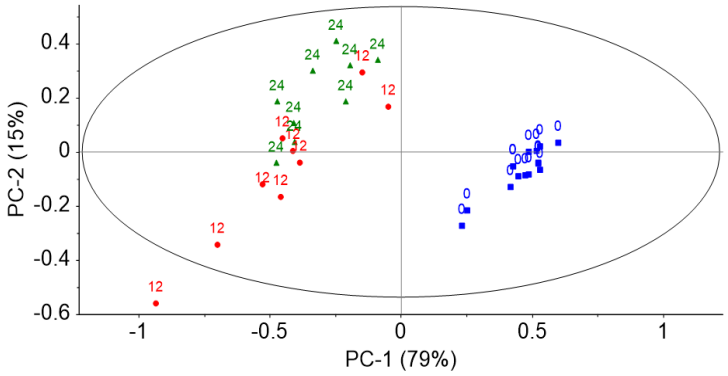

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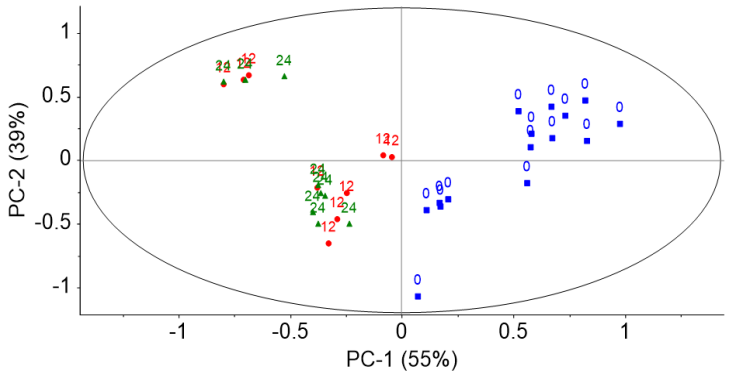

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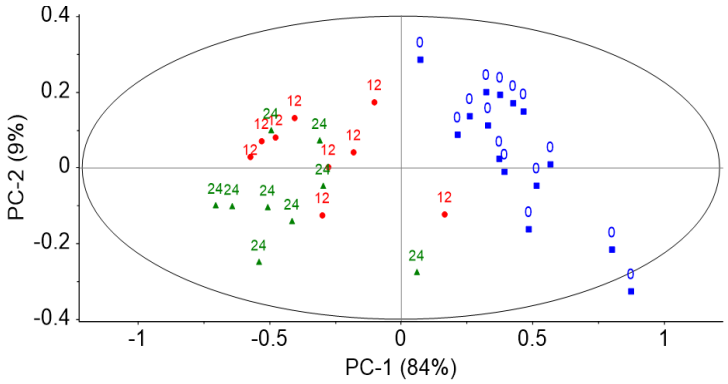
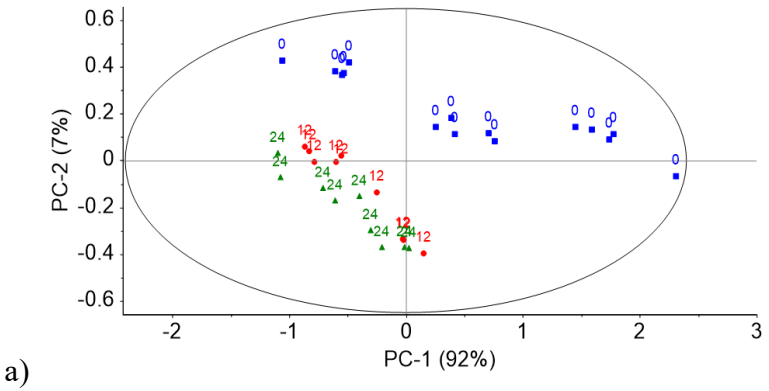


e)

Figure 4.3. PCA analysis of different faba bean partitions and faba silage in total carbohydrate region (ca. 938-1186 cm^{-1}) of (a) whole plant, (b) stem, (c) leaf, (d) whole pods and (e) whole plant faba silage. Notes: 0, 12, 24 represent original samples, 12 h incubation residue samples and 24 h incubation residue samples.



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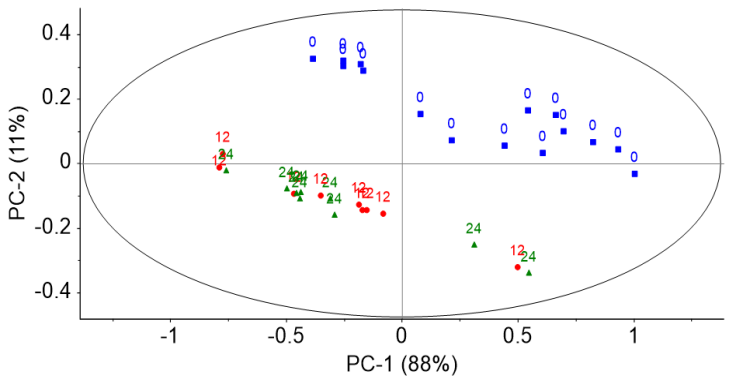

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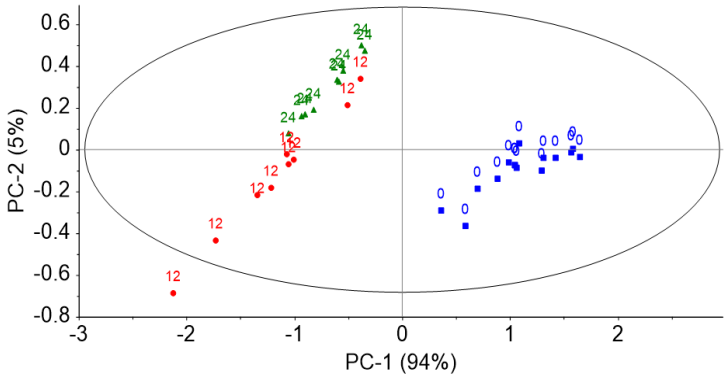
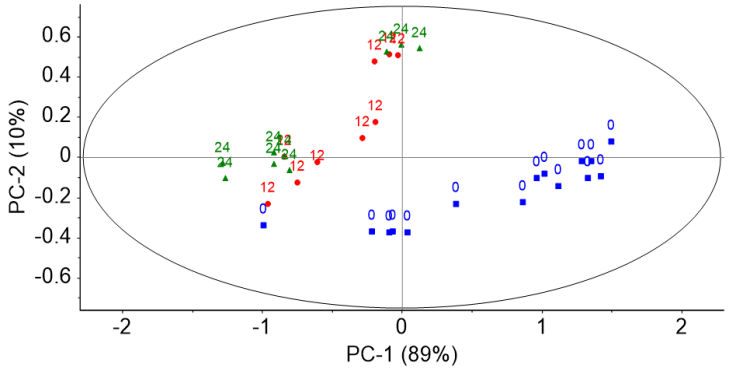
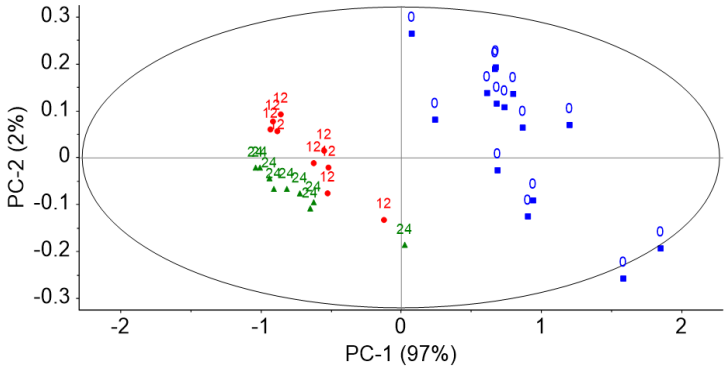


c)



d)



e)

Figure 4.4. PCA analysis of different faba bean partitions and faba silage in structural carbohydrate region (ca. 1186-1486 cm^{-1}) of (a) whole plant, (b) stem, (c) leaf, (d) whole pods and (e) whole plant silage. Notes: 0, 12, 24 represent original samples, 12 h incubation residue samples and 24 h incubation residue samples.

4.7. Chapter conclusions

Overall, mid-infrared spectrum is a comprehensive reflection of chemical compositions and molecular characteristics. Carbohydrate related spectral profiles change during incubation reveals more information about its chemical compositions while protein related spectral profiles changes tells more information about its secondary structure change which may correlated with its enzymatic accessibility.

According to the multivariate analyses, spectral profiles of original spectra of faba bean partitions and faba bean forage were separated from rumen degradation residues samples in all selected spectral regions (amide region, total carbohydrate region and structural carbohydrate region), while 12 and 24 h incubation residues were clustered together. The results demonstrated rumen degradation significantly changed protein and carbohydrate related spectral profiles in first 12 h. Chance exists in giving more detailed description about pattern of spectral change with more timepoints during rumen incubation.

According to the univariate analyses results of protein related parameters, significant interaction between faba bean samples and incubation time were observed in half of the spectral parameters, especially in the spectral ratio of α-helix to β-sheet, which is found to be correlated to protein digestibility of protein (Yu et al. 2004b). For α-helix to β-sheet, spectral ratio was increased for all faba bean samples except for WP faba bean, while during the second 12 h of rumen incubation, spectral ratios were maintained except for faba bean silage. However, protein digestibility is an overall express of comprehensive factors, which include interaction between protein and endogenous characteristics of protein. As for protein and other compound interaction, binding between protein and cell-wall structure is one of the most important reasons. As for endogenous protein factor, in addition to protein secondary structure, protein crosslink and primary structures

of γ-zein and γ-kafirin are also play important roles in determining protein digestibility (Duodu et al. 2003). As a result, whether the change of secondary structure can influence its protein availability to digestion is still uncertain and correlation and regression studies were conducted to give insight about its influence.

For carbohydrate related spectral parameters, significant interactions were observed in most of the spectral parameters except for total carbohydrate first peak (ca. 1024 cm^{-1}) peak height and peak height and peak area of cellulosic compound (ca.1304-1184 cm^{-1}). In those spectral parameters, spectral intensity was increasing with increasing incubation time which is opposite with study of Xin and Yu (2013c). In addition, in the study of Xin and Yu (2013c), almost all the spectral parameters were significantly decreased after incubation, which is not observed in this study. Detailed study needs to be done to compare the change of chemical structure during rumen digestion.

5. INTERACTIVE ASSOCIATION BETWEEN MOLECULAR STRUCTURE SPECTRAL PROFILES AND NUTRIENT UTILIZATION AND AVAILABILITY OF FABA BEAN FORAGE IN RUMINANT LIVESTOCK SYSTEM.

5.1. Abstract

The purpose of this study was to determine the internal relationship between the chemical structure information obtained through spectral analysis and nutritional and digestion characteristics acquired from wet chemistry and *in situ* rumen incubation before and after rumen degradation using different faba bean partitions and faba bean forage as reference. Firstly, correlation study was conducted with nutritional and digestion data in Chapter 3 and spectral parameters before and after rumen incubation in Chapter 4 using SAS 9.4 PROC CORR procedure. Then multi-linear regression was used to predict nutritional compositions and digestion characteristics with spectral features using PROC REG procedure with "STEPWISE" option to select the variables.

According to the results, protein related spectral profiles in original samples had strong correlation with metabolizable protein supply (r = 0.87 with α-helix) and feed milk value (r = 0.87 with α-helix) predicted with DVE/OEB system and the correlation was stronger compared with what predicted from NRC-2001 system. As for intestinal protein digestibility, strong correlation was found in original samples spectral profiles of α-helix to β-sheet (r = 0.87), however, no correlation was found after rumen degradation. As a result, we may conclude that the feed protein secondary structure has an effect to the feed undegradable protein intestinal digestibility. For protein degradation kinetics, no correlation was found between degradation rate, soluble, degradable and undegradable fractions and spectral parameters. But effective degradable protein had a strong correlation with amide I to amide II peak area ratio (r = 0.82). With regard to feed protein composition, CP, SCP and ADICP had stronger correlation with original samples spectral profiles,

NDICP had stronger correlation with incubation residue spectral profiles. For carbohydrate related chemical profiles, starch is found to have strong negative correlation with 3rd peak height of total carbohydrate (r = -0.95) in original samples spectra, while ADF had strong negative correlation with 1st peak height of structural carbohydrate in 24 h incubation residue samples spectra (r = -0.84). In addition, DVE, FMV, ECCP and starch were predicted with R^2 higher than 0.90 using both original and residue samples spectral profiles.

In conclusion, rumen degradation of carbohydrate contents can be reflected in the change of its spectral profiles. Protein availability and digestibility are mainly associated with original sample spectral profiles. Carbohydrate and protein related spectral features could be used as indicators for faba bean nutritional evaluation in dairy cattle.

5.2. Introduction

According to Yu et al.(2004b), each biological components has unique IR spectrum and chemical-structural features. For example, according to the chemical-structural feature, the characteristic of protein is unique in its peptide bond, lipid contains both carbonyl C=O ester as well as CH_2 and CH_3 functional groups, carbohydrates have lots of sugars and lots of OH and CO bonds. To link the structural feature with their specific IR spectra, we can locate their IR positions, for example accordingly, Amide I at ca. 1650 cm^{-1}, Amide II at ca. 1550 cm^{-1}, lipid at ca. 1738 cm^{-1} (carbonyl C=O), 1470 cm^{-1} (CH bending), ca. 2961 cm^{-1} (asymmetric stretch CH_3), ca. 2925 cm^{-1} (asymmetric stretch CH_2), ca. 2871 cm^{-1} (symmetric stretch CH_3), and ca. 2853 cm^{-1} (symmetric stretch CH_2), carbohydrate between ca. 1180 and 950 cm^{-1}. The same principle can be applied to other biological components.

To our knowledge, experiment in portioning faba bean whole plant to research its nutrient utilization in relation to the internal structure of plant integuments is none. The inherent structure

of feedstuffs, especially the structure of protein is vital to the understanding of the digestive behavior and nutritive value. The protein secondary structure includes α-helix, β-sheet and β-turns and random coils. According to Yu et al.(2004a), the percentage of these structures, especially the ratios of α-helixes and β-sheets, is relevant to the protein utilization of feeds. However, traditional wet chemical analysis fails to reveal the internal structure of feedstuff and to link it to the digestive characteristics. In addition, carbohydrate related spectral features can be used to determine the chemical composition of feed according to its chemical structural characteristics. Therefore, an approach capable of revealing the internal structure of feeds is needed (Yu 2005a). According to Hell et al. (2016), spectra recorded from near-infrared (NIR) range(ca. 13400–4000 cm^{-1}) are "sensitive to multitude of compounds and molecular interactions" and are capable of predicting nutrient compositions in grains. However, NIR spectra fail to identify more complex and similar structures, which could be achieved with mid-infrared spectroscopy. In addition, according to Xin and Yu (2013a), Fourier transform infrared spectroscopy (FT/IR) technique with ATR, has been successfully used to detect the structural makeups and biopolymers conformation in different feedstuffs. As a result, in order to explore the interactive association between molecular structural spectral profiles and nutrient utilization and availability of leaf, stem, pods, and whole plant Faba bean forage in ruminants FTIR-ATR technology will be used in this study.

5.3. Study objectives

To associate molecular structure spectral profiles to nutrient utilization and availability and quantify the relationship between molecular spectral profiles and nutrient utilization and availability in ruminants.

5.4. Study hypotheses

Molecular structures could have relationship with the nutrient utilization and availability and the relationship could also be found between original samples and rumen degradation as well as intestinal digestion in term of molecular spectral profiles.

5.5. Materials and Methods

5.5.1. Data preparation

For correlation and regression analyses, nutritional and digestion features of different faba bean samples (n=15) were taken from results of Chapter 3 and univariate spectral results of both original and 12 and 24 hours incubation residue samples (n=15×3) were from results of Chapter 4.

5.5.2. Correlation analysis

Nutritional and digestion data (n=15) were used for correlation study with spectral data from original (n=15) and 12 h (n=15) and 24 h (n=15) incubation residues. The correlation between protein molecular spectral features and protein rumen degradation characteristics; metabolizable protein supply and feed milk value based on DVE/OEB and NRC-2001 systems; and protein intestinal digestibility estimation were analyzed using PROC CORR procedure of SAS (version 9.4) with the FISHER option which offers confidence limits and *p* values for Pearson correlation coefficients based on Fisher's z transformation. Similarity, the Pearson correlation between chemical profiles, energy values, CNCPS parameters, NDF rumen degradation parameters and carbohydrate related spectral profiles were also conducted. All faba bean samples (stem, leaf, WP, WPL and faba silage) were used for correlation study.

5.5.3. Regression analysis

Multi-linear regression analysis of protein molecular structure profiles with rumen degradation characteristics; metabolizable protein supply and feed milk value based on DVE/OEB and NRC-2001 systems; and estimated protein intestinal digestion were carried out using the "PROC REG"

procedure of SAS (version 9.4). “STEPWISE” option was used for selecting model variables with variable selection criteria: “SLENTRY=0.05, SLSTAY=0.05”. All variables kept in the prediction model should be significant at the 0.05 level. The multi-collinearity check was carried out using an VIF option in PROC REG in SAS and variables with VIF larger than 10 were removed from the model. All faba bean samples (stem, leaf, WP, WPL and faba silage) were used for regression study.

5.6. Results and Discussion

5.6.1. Correlation study between molecular structure profiles and nutrient metabolic characteristics of protein and carbohydrates before and after rumen digestion

Feed protein consists of those soluble and readily degraded in the rumen and those cross-linked with polysaccharide, lignin, phenolic compounds and form complicated fiber matrix structure, as a result, combination of various microorganisms are needed to work together or sequentially to break down protein molecule (Wang and McAllister 2002). In addition, protein molecules with secondary structures such as β-sheet inhibit enzymatic attachment (Yu 2005b). Therefore, the protein molecular structure change during fermentation may interfere with further enzyme attachment and overall protein digestibility. However, the determination of feed nutritional value has been focused on its chemical composition, while plenty of researches have also found out the close relationship between feed molecular structure and feed nutritional availability, digestive behavior and ruminal and intestinal degradation and the relationship is variable depending on many factors such as plant variety, maturity, processing method and even gene transformation (Yu 2007; Theodoridou and Yu 2013a; Li et al. 2016; Lei et al. 2019). However, no study has been conducted to determine how spectral parameters of feed is changed during the rumen degradation and how the change is correlated to the nutritive value of the feed.

The results of correlation study of samples before and after rumen degradation are showed in Tables 5.1, 5.2 and 5.3. Protein related spectral profiles of samples before rumen incubation were found to have a strong correlation with DVE value, especially amide I and amide II peak area ratio ($r = 0.82$), α-helix to β-sheet ratio ($r = 0.85$) and α-helix peak height ($r = 0.87$). Strong positive correlation between spectral profiles and nutritional values could also be found in degraded protein balance (DPB), endogenous protein EDCP and FMV of DVE/OEB model and metabolizable protein (MP), DPB and FMV values in NRC-2001 model, except peak height ratio of amide I and amide II which was negatively correlated. In addition, α-helix to β-sheet ratio of original samples spectral profiles had a strong correlation with intestinal digestibility of rumen undegradable protein ($r = 0.87$). However, spectral parameters had poor correlation with rumen degradation kinetics parameters, except predicted EDCP, which was positively correlated with peak area and height ratios of amide I and amide II (r equals 0.82 and -0.76 respectively) and α-helix to β-sheet ratio ($r = 0.71$). In addition, chemical composition had strong correlation with original samples spectral parameters, especially α-helix to β-sheet ratio with ADICP ($r = 0.80$) and amide I to amide II ratio to SCP ($r = -0.79$).

Comparing the correlation results of samples before incubation, nutritional profiles of incubation residue samples generally had a weaker correlation relationship with spectral profiles. Especially for α-helix to β-sheet ratio, which had strong correlation with original samples nutritional profiles, turned out to have no correlation with incubation residues. However, for α-helix to β-sheet ratio, it had no correlation with NDICP for original samples, but there was a strong correlation for 12 h residue samples ($r = -0.69$) and the correlation even stronger for 24 h residue samples ($r = -0.82$). This may because with the rumen microbial digestion, neutral detergent soluble protein gradually

disappears and the proportion of NDICP begin to increase, and this decreases the interfere of other irrelevant compounds.

Table 5.1. Correlation analysis between protein structure spectral characteristics and predicted truly absorbed protein, crude protein rumen degradation parameters and protein chemical compositions of recently developed faba bean plants before rumen incubation.

	Spectral profiles peak area				Spectral profiles peak height					
Item	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
DVE/OEB model										
DVE (g/kg DM)	0.71**	0.77**	0.82**	0.71**	0.74**	0.79**	-0.77**	0.87**	0.75**	0.85**
OEB (g/kg DM)	0.58*	0.68**	0.71**	0.58*	0.57*	0.66**	-0.78**	0.60*	0.59*	0.52*
BCP (g/kg DM)	-0.15	-0.24	-0.42	-0.15	-0.16	-0.27	0.29	-0.33	-0.18	-0.63*
EDCP (g/kg DM)	0.59*	0.67**	0.73**	0.59*	0.58*	0.66**	-0.79**	0.62*	0.59*	0.55*
FMV (kg milk/kg)	0.71**	0.77**	0.82**	0.71**	0.74**	0.79**	-0.77**	0.87**	0.75**	0.85**
NRC-2001 model										
MP (g/kg DM)	0.67**	0.74**	0.84**	0.67**	0.69**	0.74**	-0.83**	0.80**	0.70**	0.82**
RUP (g/kg DM)	-0.15	-0.24	-0.42	-0.15	-0.16	-0.27	0.29	-0.33	-0.18	-0.63*
DPB (g/kg DM)	0.57*	0.67**	0.70**	0.58*	0.58*	0.66**	-0.79**	0.60*	0.59*	0.52*
FMV (kg milk/kg)	0.58*	0.66**	0.80**	0.58*	0.58*	0.68**	-0.80**	0.68*	0.60*	0.69**
Intestinal protein digestion										
dRUP (%)	0.42	0.50+	0.68**	0.42	0.46+	0.56*	-0.65**	0.65**	0.48+	0.87**
Rumen degradation parameters										
k_d (%/h)	-0.50	-0.36	-0.03	-0.50	-0.54+	-0.30	-0.29	-0.55+	-0.53+	-0.20
S (%)	0.08	0.27	0.67*	0.08	0.06	0.29	-0.69*	0.23	0.10	0.58*
D (%)	0.41	0.24	-0.16	0.41	0.45	0.16	0.46	0.43	0.44	0.10
Predicted EDCP (%)	0.24	0.40	0.82**	0.24	0.26	0.41	-0.76**	0.43	0.29	0.71**
Protein profiles										
CP (%DM)	0.59*	0.68**	0.70**	0.59*	0.58*	0.67**	-0.76**	0.61*	0.59*	0.51+
NDICP (%DM)	0.74**	0.69**	0.26	0.74**	0.70**	0.62*	-0.18	0.61*	0.70**	0.18
ADICP (%DM)	-0.40	-0.48+	-0.73**	-0.40	-0.43	-0.51+	0.64*	-0.59*	-0.45+	0.80**
SCP (%DM)	0.61*	0.69**	0.76**	0.61*	0.61*	0.69**	-0.79**	0.71**	0.63*	0.75**

Notes: DVE: truly digested protein in the small intestine; OEB: degraded protein balance; MP: metabolizable protein; BCP: bypass crude protein; EDCP: effective degradability of CP; FMV: feed milk value; MP: metabolizable protein; RUP; rumen undegraded feed protein; DPB: rumen degraded protein balance; dRUP: intestinal digestibility of rumen undegraded protein; k_d: rate of degradation; S: soluble fraction in the in situ incubation; D: potential degradable fraction; EDCP: effective degraded crude protein; CP: crude protein; NDICP: neutral detergent insoluble crude

protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; "+": $P < 0.10$, not significant with tendency; "*": $P < 0.05$, significant; "**": $P < 0.01$, strongly significant. r: correlation coefficient using spearman method.

Table 5.2. Correlation analysis between protein structure spectral characteristics and predicted truly absorbed protein, crude protein rumen degradation parameters and protein chemical compositions of recently developed faba bean plants after 12 h incubation.

	Spectral profiles peak area				Spectral profiles peak height					
Item	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
DVE/OEB model										
DVE (g/kg DM)	0.66**	0.72**	-0.47^{+}	0.66**	0.68**	0.78**	-0.58*	0.65**	0.65**	-0.32
OEB (g/kg DM)	0.54*	0.50^{+}	-0.16	0.54*	0.54*	0.55*	-0.38	0.54*	0.54*	-0.36
BCP (g/kg DM)	-0.04	-0.14	0.08	-0.04	-0.06	-0.22	0.09	-0.04	-0.04	-0.46^{+}
EDCP (g/kg DM)	0.50^{+}	0.48^{+}	-0.26	0.50^{+}	0.49^{+}	0.53*	-0.42	0.49^{+}	0.49^{+}	-0.27
FMV (kg milk/kg)	0.66**	0.72**	-0.47^{+}	0.66**	0.68**	0.78**	-0.58*	0.65**	0.65**	-0.32
NRC-2001 model										
MP (g/kg DM)	0.54*	0.61*	-0.50^{+}	0.54*	0.56*	0.67**	-0.62*	0.53*	0.53*	-0.19
RUP (g/kg DM)	-0.04	-0.14	0.08	-0.04	-0.06	-0.22	0.09	-0.04	-0.04	-0.46^{+}
DPB (g/kg DM)	0.53*	0.49^{+}	-0.21	0.53*	0.52*	0.54*	-0.38^{+}	0.52*	0.52*	-0.34
FMV (kg milk/kg)	0.48^{+}	0.52*	-0.47^{+}	0.48+	0.49^{+}	0.59*	-0.55*	0.47^{+}	0.47^{+}	-0.17
Intestinal protein digestion										
dRUP (%)	0.36	0.49^{+}	-0.56*	0.36	0.39	0.56*	-0.58*	0.35	0.35	0.03
Rumen degradation parameters										
k_d (%/h)	-0.25	-0.34	0.29	-0.25	-0.29	-0.38	0.33	-0.23	-0.23	0.13
S (%)	0.04	0.06	-0.08	0.04	0.03	0.16	-0.05	0.03	0.03	0.47
D (%)	0.17	0.28	-0.31	0.17	0.21	0.31	-0.42	0.19	0.19	-0.18
Predicted EDCP (%)	0.00	0.08	-0.25	0.00	0.02	0.21	-0.21	0.00	0.00	0.54^{+}
Protein profiles										
CP (%DM)	0.53*	0.49^{+}	-0.17	0.53*	0.52*	0.54*	-0.39	0.52*	0.52*	-0.35
NDICP (%DM)	0.76**	0.68**	0.05	0.76**	0.73**	0.66**	-0.30	0.76**	0.76**	-0.69**
ADICP (%DM)	-0.24	-0.35	0.52*	-0.24	-0.27	-0.45^{+}	0.49^{+}	-0.24	-0.24	-0.19
SCP (%DM)	0.51^{+}	0.56*	-0.45^{+}	0.51^{+}	0.52*	0.62*	-0.63*	0.51^{+}	0.51^{+}	-0.18

Notes: DVE: truly digested protein in the small intestine; OEB: degraded protein balance; MP: metabolizable protein; BCP: bypass crude protein; EDCP: effective degradability of CP; FMV: feed milk value; MP: metabolizable protein; RUP; rumen undegraded feed protein; DPB: rumen degraded protein balance; dRUP: intestinal digestibility of rumen undegraded protein; k_d: rate of degradation; S: soluble fraction in the *in situ* incubation; D: potential degradable fraction; EDCP: effective degraded crude protein; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; "+": $P < 0.10$, not significant with tendency; "*": $P < 0.05$, significant; "**": $P < 0.01$, strongly significant. r: correlation coefficient using spearman method.

Table 5.3. Correlation analysis between protein structure spectral characteristics and predicted truly absorbed protein, crude protein rumen degradation parameters and protein chemical compositions of recently developed faba bean plants after 24 h incubation.

Item	spectral profiles peak area				spectral profiles peak height					
	amide I	amide II	amide I to amide II	total amide	amide I	amide II	amide I to amide II	α-helix	β-sheet	α-helix to β-sheet
DVE/OEB model										
DVE (g/kg DM)	0.60*	0.59*	-0.04	0.61*	0.61*	0.43	-0.32	0.60*	0.59*	-0.46^{+}
OEB (g/kg DM)	0.62*	0.64*	-0.08	0.64*	0.63*	0.48^{+}	-0.27	0.62*	0.62*	-0.38
BCP (g/kg DM)	0.02	0.06	-0.40	0.06	0.05	0.24	-0.25	0.03	0.03	-0.21
EDCP (g/kg DM)	0.56*	0.57*	-0.08	0.57*	0.57*	0.41	-0.30	0.56*	0.55*	-0.34
FMV (kg milk/kg)	0.60*	0.59*	-0.04	0.61*	0.61*	0.43	-0.32	0.60*	0.59*	-0.46^{+}
NRC-2001 model										
MP (g/kg DM)	0.53*	0.49^{+}	-0.03	0.52*	0.52*	0.35	-0.33	0.52*	0.51*	-0.34
RUP (g/kg DM)	0.02	0.06	-0.40	0.06	0.05	0.24	-0.25	0.03	0.03	-0.21
DPB (g/kg DM)	0.59*	0.63*	-0.15	0.62*	0.61*	0.48^{+}	-0.34	0.60*	0.60*	-0.38
FMV (kg milk/kg)	0.47^{+}	0.47^{+}	-0.09	0.49^{+}	0.48^{+}	0.30	-0.29	0.47^{+}	0.46^{+}	-0.29
Intestinal protein digestion										
dRUP (%)	0.28	0.25	0.04	0.27	0.27	0.08	-0.20	0.28	0.27	-0.14
Rumen degradation parameters										
k_d (%/h)	-0.01	0.09	-0.25	0.03	0.00	-0.09	0.18	-0.01	0.00	0.22
S (%)	0.06	0.12	0.22	0.09	0.08	-0.25	0.25	0.06	0.06	0.29
D (%)	-0.03	-0.18	0.32	-0.11	-0.08	0.07	-0.28	-0.03	-0.02	-0.24
Predicted EDCP (%)	0.01	0.05	0.22	0.02	0.03	-0.30	0.09	0.01	0.00	0.37
Protein profiles										
CP (%DM)	0.60*	0.62*	-0.10	0.62*	0.61*	0.47^{+}	-0.31	0.61*	0.60*	-0.37
NDICP (%DM)	0.72**	0.62*	0.17	0.68**	0.69**	0.74**	-0.27	0.71**	0.71**	-0.82**
ADICP (%DM)	-0.18	-0.15	-0.13	-0.16	-0.17	0.05	0.09	-0.17	-0.16	0.00
SCP (%DM)	0.52*	0.47^{+}	0.08	0.50^{+}	0.51^{+}	0.32	-0.28	0.52*	0.52*	-0.33

Notes: DVE: truly digested protein in the small intestine; OEB: degraded protein balance; MP: metabolizable protein; BCP: bypass crude protein; EDCP: effective degradability of CP; FMV: feed milk value; MP: metabolizable protein; RUP; rumen undegraded feed protein; DPB: rumen degraded protein balance; dRUP: intestinal digestibility of rumen undegraded protein; k_d: rate of degradation; S: soluble fraction in the *in situ* incubation; D: potential degradable fraction; EDCP: effective degraded crude protein; CP: crude protein; NDICP: neutral detergent insoluble crude

protein; ADICP: acid detergent insoluble crude protein; SCP: soluble crude protein; "+": $P < 0.10$, not significant with tendency; "*": $P < 0.05$, significant; "**": $P < 0.01$, strongly significant. r: correlation coefficient using spearman method.

The change of spectral profiles during rumen incubation reflect more information on ruminal degradation to change of carbohydrate fractions and could probably associated with not only nutrient availability but also digestive characteristics. Therefore, correlation between nutritional and spectral profiles was conducted for both original and incubation residue samples; and the results are presented in Tables 5.4, 5.5 and 5.6. As for chemical composition, spectral profiles of original samples have stronger correlation with starch content, especially peak area of structural carbohydrate (r = -0.88), 2nd peak height of structural carbohydrate (r = -0.92) and 3rd peak height of total carbohydrate (r = -0.95). In incubation residue samples the correlation between starch and spectral parameters was weaker. For structural carbohydrate contents (NDF, ADF, ADL, hemicellulose and cellulose), correlation was stronger for residue samples spectral profiles. As for CNCPS fractions, unavailable carbohydrate fraction of CC had no or weak correlation with original samples spectral profiles but was found to have strong correlation with incubation residue spectral profiles. However, for other chemical fractions, they had stronger correlation with their spectral data in original samples. In terms of rumen degradation parameters, stronger correlation with digestion rate, potential degradable fraction and effective degradable NDF was found with increasing incubation time. In general, the spectral parameters correlated with nutritional profiles were different between original samples and incubation residues samples. In addition, incubation residue samples spectral profiles have stronger correlation with rumen degradation parameters, and with less degradable carbohydrate fractions, while original samples spectral profiles have stronger correlation with easily degradable carbohydrate fraction.

Table 5.4. Correlation analyses between carbohydrates structure spectral characteristics and chemical profiles, carbohydrates sub-fractions, estimated energy value and rumen degradation parameters of recently developed faba bean plants before rumen incubation.

	Structural carbohydrates (STCHO)				Cellulosic compounds (CEC)		Total carbohydrates (TCHO)					Spectral ratios		
Item	area	1st peak	2nd peak	3rd peak	peak	area	area	1st peak	2nd peak	3rd peak	4th peak	STCHO: TCHO	CEC: TCHO	CEC: STCHO
Basic nutrient profiles (%DM)														
NDF	0.21	0.11	0.25	-0.72**	0.55*	0.70**	0.42	0.60*	-0.20	0.28	-0.44^{+}	0.10	-0.68**	-0.47^{+}
ADF	0.21	0.21	0.26	-0.78**	0.43	0.59*	0.30	0.45^{+}	-0.30	0.29	-0.61*	0.01	-0.62*	-0.38
ADL	0.70**	0.55*	0.76**	-0.23	0.13	0.23	0.63*	0.28	0.31	0.81**	-0.60*	-0.34	-0.25	-0.03
hemicellulose	0.12	-0.18	0.14	-0.55*	0.73**	0.84**	0.48^{+}	0.61*	-0.06	0.15	-0.06	0.32	-0.72**	-0.60*
cellulose	0.07	0.07	0.12	-0.83**	0.55*	0.69**	0.29	0.54*	-0.35	0.15	-0.53*	0.15	-0.73**	-0.51*
starch	-0.88**	-0.73**	-0.92**	-0.16	0.21	0.13	-0.63*	-0.13	-0.58*	-0.95**	0.49^{+}	0.57*	-0.14	-0.30
Carbohydrate sub-fractions (%DM)														
CA4	0.65**	0.26	0.60*	0.63*	-0.09	-0.13	0.64*	0.06	0.92**	0.64*	0.23	-0.26	0.20	0.17
CB1	-0.88**	-0.73**	-0.92**	-0.16	0.21	0.13	-0.63*	-0.13	-0.58*	-0.95**	0.49^{+}	0.57*	-0.14	-0.30
CB2	0.65**	0.68**	0.62*	0.46^{+}	-0.81**	-0.70**	-0.13	-0.51*	0.30	0.56*	-0.42	-0.86**	0.70**	0.85**
CB3	-0.28	-0.12	-0.18	-0.78**	0.52^{+}	0.66**	0.04	0.57*	-0.58*	-0.22	-0.30	0.35	-0.66**	-0.56*
CC	0.29	0.24	0.32	-0.62*	0.43	0.54^{+}	0.41	0.47	-0.14	0.34	-0.46	-0.04	-0.54^{+}	-0.33
Estimated energy values														
tdNFC (%DM)	-0.36	-0.36	-0.45^{+}	0.64*	-0.36	-0.49^{+}	-0.53*	-0.54*	0.08	-0.50^{+}	0.60*	0.03	0.51^{+}	0.31
tdNDF (%DM)	0.49^{+}	0.28	0.56*	-0.44^{+}	0.35	0.46^{+}	0.66**	0.54*	0.15	0.58*	-0.44^{+}	0.01	-0.45^{+}	-0.29
TDN_{1x} (Mcal/kg)	-0.68**	-0.52*	-0.74**	0.25	-0.16	-0.26	-0.62*	-0.29	-0.28	-0.79**	0.58*	0.32	0.29	0.06
Rumen degradation parameters														
k_d (%/h)	0.37	0.11	0.37	0.42	-0.41	-0.43	0.16	-0.20	0.41	0.36	0.07	-0.20	0.45^{+}	0.40
D (%)	-0.32	-0.26	-0.38	0.68**	-0.30	-0.42	-0.31	-0.33	0.22	-0.40	0.67**	0.08	0.43	0.21
EDNDF (%)	0.28	0.12	0.23	0.83**	-0.61*	-0.71**	-0.02	-0.44	0.56*	0.24	0.34	-0.31	0.72**	0.59*

Notes: NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CA4 = sugar (rapidly degradable carbohydrate fraction); CB1 = starch (intermediately degradable carbohydrate fraction); CB2 = soluble fiber (intermediately degradable carbohydrate fraction); CB3 =

digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction); CC = indigestible fiber (unavailable neutral detergent fiber); tdNFC: truly digestible non-fiber carbohydrates; tdNDF: truly digestible neutral detergent fiber; $TDN_{1\times}$: total digestible nutrient at one time maintenance; k_d: rate of degradation; D: potential degradable fraction in the *in situ* incubation; EDNDF: effective degraded neutral; "+": $P < 0.10$, not significant with tendency; "*": $P < 0.05$, significant; "**": $P < 0.01$, strongly significant. r: correlation coefficient using spearman method.

Table 5.5. Correlation analyses between carbohydrates structure spectral characteristics and chemical profiles, carbohydrates sub-fractions, estimated energy value and rumen degradation parameters of recently developed faba bean plants 12 h incubation residue samples.

	Structural carbohydrates (STCHO)				Cellulosic compounds (CEC)		Total carbohydrates (TCHO)					Spectral ratios		
Item	area	1st peak	2nd peak	3rd peak	peak	area	area	1st peak	2nd peak	3rd peak	4th peak	STCHO: TCHO	CEC: TCHO	CEC: STCHO
Basic nutrient profiles (%DM)														
NDF	0.63*	0.62*	0.44^{+}	0.66**	-0.19	-0.24	-0.12	-0.37	0.24	0.52*	0.24	0.81**	-0.27	-0.57*
ADF	-0.42	-0.72**	-0.48^{+}	-0.35	0.39	0.42	0.34	0.48^{+}	-0.31	-0.61*	0.01	-0.81**	0.43	0.77**
ADL	-0.41	-0.70**	-0.45^{+}	-0.42	0.43	0.44^{+}	0.32	0.48^{+}	-0.32	-0.65**	0.04	-0.81**	0.47^{+}	0.81**
hemicellulose	-0.84**	-0.57*	-0.53*	-0.80**	0.05	0.08	-0.10	0.18	-0.01	-0.41	-0.42	-0.78**	0.10	0.41
cellulose	-0.28	-0.76**	-0.40	-0.18	0.41	0.42	0.48^{+}	0.49^{+}	-0.17	-0.44	0.14	-0.73**	0.36	0.61*
starch	-0.29	-0.64**	-0.37	-0.38	0.54*	0.56*	0.39	0.55*	-0.26	-0.60*	0.07	-0.74**	0.58*	0.85**
Carbohydrate sub-fractions (%DM)														
CA4	-0.58*	-0.03	-0.26	-0.42	-0.38	-0.38	-0.46^{+}	-0.28	0.35	0.25	-0.58*	-0.08	-0.41	-0.40
CB1	-0.90**	0.35	0.52*	0.69**	0.33	0.31	0.41	0.14	0.03	0.25	0.56*	0.54*	0.26	-0.03
CB2	-0.49^{+}	0.11	-0.42	-0.03	-0.61*	-0.65**	-0.54*	-0.53*	-0.11	-0.07	-0.27	-0.04	-0.60*	-0.28
CB3	0.04	-0.48^{+}	-0.20	0.03	0.40	0.42	0.41	0.39	-0.48^{+}	-0.47^{+}	0.25	-0.42	0.46^{+}	0.60*
CC	-0.48^{+}	-0.65*	-0.56*	-0.28	0.24	0.30	0.25	0.40	-0.40	-0.61*	-0.11	-0.80**	0.33	0.75**
Estimated energy values														
tdNFC (%DM)	0.58*	0.63*	0.44	0.59*	-0.24	-0.29	-0.15	-0.39	0.30	0.57*	0.20	0.79**	-0.34	-0.63*
tdNDF (%DM)	-0.60*	-0.64*	-0.48^{+}	-0.48^{+}	0.15	0.14	0.16	0.30	-0.24	-0.59*	-0.19	-0.79**	0.16	0.46^{+}
TDN_{1x} (Mcal/kg)	0.82**	0.55*	0.52*	0.78**	-0.07	-0.10	0.08	-0.19	-0.01	0.43	0.41	0.77**	-0.13	-0.43
Rumen degradation parameters														
k_d (%/h)	-0.22	0.25	-0.21	0.16	-0.50^{+}	-0.59*	-0.24	-0.40	-0.02	-0.11	-0.23	0.01	-0.60*	-0.49^{+}
D (%)	0.39	0.68**	0.33	0.42	-0.41	-0.40	-0.39	-0.49^{+}	0.22	0.68**	-0.14	0.82**	-0.41	-0.76**
EDNDF (%)	-0.13	0.59*	-0.05	0.20	-0.71**	-0.77**	-0.58*	-0.70**	0.18	0.30	-0.40	0.47^{+}	-0.77**	-0.85**

Notes: NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CA4 = sugar (rapidly degradable carbohydrate fraction); CB1 = starch (intermediately degradable carbohydrate fraction); CB2 = soluble fiber (intermediately degradable carbohydrate fraction); CB3 = digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction); CC = indigestible fiber (unavailable neutral detergent

fiber); tdNFC: truly digestible non-fiber carbohydrates; tdNDF: truly digestible neutral detergent fiber; $TDN_{1\times}$: total digestible nutrient at one time maintenance; k_d: rate of degradation; D: potential degradable fraction in the *in situ* incubation; EDNDF: effective degraded neutral; "+": $P < 0.10$, not significant with tendency; "*": $P < 0.05$, significant; "**": $P < 0.01$, strongly significant. r: correlation coefficient using spearman method.

Table 5.6. Correlation analyses between carbohydrates structure spectral characteristics and chemical profiles, carbohydrates sub-fractions, estimated energy value and rumen degradation parameters of recently developed faba bean plants 24 h incubation residue samples.

	Structural carbohydrates (STCHO)				Cellulosic compounds (CEC)	Total carbohydrates (TCHO)					Spectral ratios		
Item	area	1st peak	2nd peak	3rd peak	peak	area	area	1st peak	2nd peak	3rd peak	4th peak	STCHO: TCHO	CEC: TCHO	CEC: STCHO
Basic nutrient profiles (%DM)														
NDF	0.66**	0.70**	0.67**	0.76**	-0.31	-0.33	-0.10	-0.27	-0.02	0.22	0.25	0.51+	-0.40	-0.63*
ADF	-0.53*	-0.84**	-0.51+	-0.52*	0.50+	0.56*	0.34	0.49+	0.12	-0.20	0.02	-0.70**	0.48+	0.81**
ADL	-0.59*	-0.85**	-0.55*	-0.60*	0.48+	0.54*	0.32	0.49+	0.16	-0.18	-0.01	-0.68**	0.47+	0.81**
hemicellulose	-0.54*	-0.48+	-0.58*	-0.65**	0.13	0.20	-0.11	0.02	-0.02	-0.15	-0.35	-0.29	0.31	0.43
cellulose	-0.32	-0.76**	-0.34	-0.33	0.60*	0.59*	0.52*	0.57*	0.22	-0.04	0.30	-0.70**	0.43	0.72**
starch	-0.51+	-0.82**	-0.47+	-0.56*	0.58*	0.61*	0.39	0.56*	0.27	-0.10	0.05	-0.65**	0.54*	0.82**
Carbohydrate sub-fractions (%DM)														
CA4	-0.06	0.35	-0.18	-0.13	-0.24	-0.28	-0.42	-0.35	-0.09	0.05	-0.43	0.33	-0.10	-0.31
CB1	0.53*	0.17	0.59*	0.55*	0.24	0.16	0.41	0.28	0.29	0.26	0.58*	0.06	-0.03	-0.11
CB2	-0.25	0.25	-0.28	0.04	-0.74**	-0.55*	-0.56*	-0.49+	-0.56*	-0.33	-0.53*	0.16	-0.47+	-0.29
CB3	-0.23	-0.69**	-0.16	-0.25	0.49+	0.47+	0.42	0.49+	0.09	-0.13	0.20	-0.59*	0.36	0.61*
CC	-0.63*	-0.79**	-0.63*	-0.57*	0.38	0.45	0.24	0.44	0.04	-0.31	-0.12	-0.71**	0.36	0.80**
Estimated energy values														
tdNFC (%DM)	0.64*	0.74**	0.63*	0.72**	-0.36	-0.38	-0.13	-0.31	-0.03	0.24	0.22	0.54*	-0.43	-0.68**
tdNDF (%DM)	-0.71**	-0.67**	-0.72**	-0.75**	0.28	0.23	0.17	0.33	-0.01	-0.25	-0.20	-0.59*	0.24	0.62*
TDN_{1x} (Mcal/kg)	0.52*	0.49+	0.56*	0.63*	-0.13	-0.21	0.09	-0.02	0.02	0.16	0.34	0.30	-0.31	-0.45+
Rumen degradation parameters														
k_d (%/h)	-0.25	0.28	-0.32	-0.06	-0.60*	-0.68**	-0.24	-0.25	-0.47+	-0.31	-0.36	0.03	-0.66**	-0.33
D (%)	0.71**	0.85**	0.66**	0.72**	-0.42	-0.48+	-0.37	-0.48+	-0.18	0.17	-0.09	0.73**	-0.39	-0.82**
EDNDF (%)	0.21	0.77**	0.13	0.37	-0.81**	-0.86**	-0.57*	-0.64*	-0.53*	-0.18	-0.46+	0.56*	-0.73**	-0.79**

Notes: NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CA4 = sugar (rapidly degradable carbohydrate fraction); CB1 = starch (intermediately degradable carbohydrate fraction); CB2 = soluble fiber (intermediately degradable carbohydrate fraction); CB3 = digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction); CC = indigestible fiber (unavailable neutral detergent fiber); tdNFC: truly digestible non-fiber carbohydrates; tdNDF: truly digestible neutral detergent fiber; $TDN_{1\times}$: total digestible nutrient at one time maintenance; k_d: rate of degradation; D: potential degradable fraction in the *in situ* incubation; EDNDF: effective degraded neutral; "+": $P < 0.10$, not significant with tendency; "*": $P < 0.05$, significant; "**": $P < 0.01$, strongly significant. r: correlation coefficient using spearman method.

5.6.2. Regression study between molecular structure profiles and nutrient metabolic characteristics of protein and carbohydrates before and after rumen digestion

Predictions of feed protein compositions and digestion characteristics have been reported in many studies (Li et al. 2016; Lei et al. 2019), however, none of these studies has used the spectral profiles of incubation residue samples. In this study, both the spectral profiles of original and incubation residues samples were used to predict the protein related nutritive values and the results are presented in Tables 5.7, 5.8 and 5.9. Compared with NRC-2001 model, spectral data gave better prediction of parameters in DVE/OEB model. For DVE/OEB system, DVE, EDCP and FMV were precisely predicted with the R^2 greater than 0.90; α-helix to β-sheet ratio were included in all these predictions. This may indicate the inherent relationship between feed protein digestion characteristics and α-helix to β-sheet ratio of incubation residue samples. As for feed protein contents, NDICP was predicted with very strong estimation power (R^2 = 0.85). As considerable proportion of feed protein is presented in cell wall structure (Tan et al. 2013), chance may exist in giving precise prediction of cell wall bonded protein fractions by including carbohydrate related spectral profiles.

Carbohydrate related spectral profiles were also used for prediction and the results were shown in Tables 5.10 and 5.11. Carbohydrate related spectral profiles were found to be able to predict carbohydrate related nutrients with R^2 greater than 0.85, except for hemicellulose (R^2 = 0.72) and CNCPS CB3 fraction (R^2 = 0.32). However, NDF degradation kinetics parameters were only predicted with R^2 less than 0.70.

Table 5.7. Multiple regression analysis to choose the most important protein spectral parameters for predicting predicted truly absorbed protein, intestinal protein degradation, crude protein rumen degradation parameters of recently developed faba bean plants.

predicted variable (y)	variable selection ($P < 0.05$)	prediction equation $Y = a + b_1 \times x_1 + b_2 \times x_2 + \cdots + b_n \times x_n$	model R^2	RSD	P value
DVE-OEB model					
DVE (g/kg DM)	HAII12, alpha_beta24	DVE (g/kg DM) = 1207.7 HAII12 + 228.7 alpha_beta24 - 310.8	0.94	8.06	<0.001
OEB (g/kg DM)	alpha_beta24, AAII12	OEB (g/kg DM) = 466.6 alpha_beta24 + 41.75 AAII12 - 684.3	0.79	26.46	<0.001
BCP (g/kg DM)	alpha_beta	BCP (g/kg DM) = -18.87 alpha_beta + 53.13	0.33	3.00	0.0255
EDCP (g/kg DM)	alpha_beta24, AAI12, HAI_AII12	EDCP (g/kg DM) = 890.5 alpha_beta24 + 22.0 AAI12 - 26.6 HAI_AII12 - 1106.9	0.92	23.92	<0.001
FMV (kg milk/kg)	alpha_beta24, HAII12	FMV (kg milk/kg) = 24.53 HAII12 + 4.65 alpha_beta24 - 6.32	0.95	0.16	<0.001
NRC values					
MP (g/kg DM)	HAI_AII	MP (g/kg DM) = -75.45 HAI_AII + 182.21	0.61	14.25	<0.001
RUP (g/kg DM)	alpha_beta	RUP (g/kg DM) = -17.00 alpha_beta + 47.86	0.33	2.70	0.0255
DPB (g/kg DM)	HAI_AII	DPB (g/kg DM) = -231.85 HAI_AII + 402.78	0.64	40.54	<0.001
FMV (kg milk/kg)	HAI_AII	FMV (kg milk/kg) = -1.53 HAI_AII + 3.70	0.61	0.29	<0.001
Intestinal protein digestion					
dRUP (%)	alpha_beta	dRUP (%) = 147.49 alpha_beta - 79.56	0.73	10.03	<0.001
Rumen degradation parameters					
S (%)	AAI_AII	S (%) = 71.83 AAI_AII + 30.42	0.52	3.43	0.008
EDCP (%)	alpha_beta	EDCP (%) = 30.94 alpha-beta + 53.76	0.68	2.01	0.001

Notes: DVE: truly digested protein in the small intestine; OEB: degraded protein balance; BCP: bypass crude protein; EDCP: effective degradability of CP; FMV: feed milk value; MP: metabolizable protein; RUP; rumen undegraded feed protein; DPB: rumen degraded protein balance; dRUP: intestinal digestibility of rumen undegraded protein; S: soluble fraction in the *in situ* incubation; HAII12: 12h incubation residue amide II peak height; alpha_beta24: 24h incubation residue α-helix to β-sheet peak height ratio; AAII12: 12h incubation residue amide II peak area; HAI_AII12: 12h incubation residue amide I to amide II peak height ratio; HAII: amide II peak height; HAI_AII: amide I to amide II peak height ratio; alpha_beta: α-helix to β-sheet peak height ratio; AAI_AII: amide I to amide II peak area ratio; RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 level.

Table 5.8. Multiple regression analysis to choose the most important protein spectral parameters for predicting protein profiles, estimated energy profiles of recently developed faba bean plants.

predicted variable (y)	variable selection ($P < 0.05$)	prediction equation $Y = a + b_1 \times x_1 + b_2 \times x_2 + \cdots + b_n \times x_n$	model R^2	RSD	P value
Protein profiles					
CP (%DM)	HAI_AII	CP (%DM) = -26.91 HAI_AII + 58.44	0.64	4.78	<0.001
NDICP (%DM)	HAI, HAI_AII, alpha_beta, AAI_AII	NDICP (%DM) = 9.29 HAI – 2.53 HAI_AII - 4.88 alpha_beta – 16.74 AAI_AII + 12.24	0.85	0.38	<0.001
SCP (%DM)	alpha_beta	SCP (%DM) = 44.89 alpha_beta - 32.40	0.68	3.45	<0.001
NDICP (%CP)	AAI_AII	NDICP (%CP) = -63.71 AAI_AII + 28.01	0.42	3.96	0.009
ADICP (%CP)	alpha_beta	ADICP (%CP) = -16.61 alpha_beta + 18.76	0.57	1.61	0.001
Estimated energy values					
tdCP (%DM)	HAI_AII	tdCP (%DM) = -27.18 HAI_AII + 58.46	0.64	4.79	<0.001
TDN_{1x} (Mcal/kg)	alpha, HAI_AII	TDN_{1x} (Mcal/kg) = 33.68 alpha - 22.18 HAI_AII + 88.42	0.75	5.32	<0.001
DE_{1x} (Mcal/kg)	HAI_AII, alpha	DE_{1x} (Mcal/kg) = 1.76 alpha - 1.18 HAI_AII + 4.22	0.75	0.28	<0.001
DEp_{3x} (Mcal/kg)	HAI_AII	DEp_{3x} (Mcal/kg) = -1.45 HAI_AII + 4.96	0.65	0.25	<0.001
ME_dairy (Mcal/kg)	HAI_AII, alpha	ME_dairy (Mcal/kg) = 1.79 alpha - 1.19 HAI_AII + 3.81	0.75	0.28	<0.001
$NEL_{\_3X}$ (Mcal/kg)	HAI_AII	$NEL_{\_3X}$ (Mcal/kg) = -0.85 HAI_AII + 2.88	0.64	0.15	<0.001
ME_beef (Mcal/kg)	alpha, HAI_AII	ME_beef (Mcal/kg) = 1.44 alpha - 0.97 HAI_AII + 3.46	0.75	0.23	<0.001
NEm (Mcal/kg)	HAI_AII	NEm (Mcal/kg) = -1.37 HAI_AII + 3.60	0.65	0.24	<0.001
NEg (Mcal/kg)	HAI_AII	NEg (Mcal/kg) = -1.24 HAI_AII + 2.79	0.65	0.21	<0.001

Note: DM: dry matter; CP: crude protein; NDICP: neutral detergent insoluble crude protein; ADICP: acid detergent insoluble crude protein; tdCP: truly digestible crude protein; $TDN_{1\times}$: total digestible nutrient at one time maintenance. $DE_{1\times}$: digestible energy at production level of intake (1×); ME: metabolizable energy at production level of intake; $NEL_{3\times}$: net energy for lactation at production level of intake (3×); NEm: net energy for maintenance; NEg: net energy for growth; HAI_AII: amide I to amide II peak height ratio; beta: β-sheet peak height; HAI: amide I peak height; alpha: α-helix peak height; alpha_beta: α-helix to β-sheet peak height ratio; AAI_AII: amide I to amide II peak area ratio ;RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 level.

Table 5.9. Multiple regression analysis to choose the most important protein spectral parameters for predicting protein sub-fraction of recently developed faba bean plants.

predicted variable (y)	variable selection ($P < 0.05$)	prediction equation $Y = a + b_1 \times x_1 + b_2 \times x_2 + \cdots + b_n \times x_n$	model R^2	RSD	P value
Protein sub-fractions					
PA2 (%CP)	alpha_beta	PA2 (%CP) = 67.79 alpha_beta - 5.74	0.62	5.87	<0.001
PB1 (%CP)	alpha_beta	PB1 (%CP) = -71.49 alpha_beta + 105.31	0.65	5.89	<0.001
PB2 (%CP)	AAI, AAI_AII	PB2 (%CP) = 0.17 AAI – 50.72 AAI_AII + 16.55	0.53	2.18	0.01
PC (%CP)	alpha_beta	PC (%CP) = -16.61 alpha_beta + 18.76	0.57	1.61	0.001
TP (%CP)	alpha_beta	TP (%CP) = -16.19 alpha_beta + 118.19	0.48	1.86	0.004
PA2 (%TP)	alpha_beta	PA2 (%TP) = 0.76 alpha_beta - 0.15	0.65	0.06	<0.001
PB1 (%TP)	alpha_beta	PB1 (%TP) = -0.65 alpha_beta + 0.98	0.63	0.06	<0.001
Predicted RDP					
RDPA2 (% of DM)	alpha_beta	RDPA2 (% of DM) = 38.26 alpha_beta - 27.36	0.68	2.94	<0.001
RDPB1 (% of DM)	AAI, alpha	RDPB1 (% of DM) = 0.29 AAI - 17.40 alpha + 0.28	0.63	1.09	0.003
RDPB2 (% of DM)	beta, AAI_AII	RDPB2 (% of DM) = 4.09 beta – 5.51 AAI_AII + 1.10	0.67	0.25	0.001
RDP (% of DM)	HAII	RDP (% of DM) = 53.95 HAII + 3.41	0.67	3.62	<0.001
Predicted RUP					
RUPA2 (% of DM)	alpha_beta	RUPA2 (% of DM) = 6.72 alpha_beta - 5.16	0.69	0.50	<0.001
RUPB2 (% of DM)	HAII, AAI	RUPB2 (% of DM) = -2.14 HAII + 0.03AAI - 0.12	0.92	0.06	<0.001
RUP (% of DM)	HAI_AII	RUP (% of DM) = -5.70 HAI_AII + 12.52	0.59	1.12	<0.001

Notes: PA2: rapidly degradable protein fraction; PB1: moderately degradable protein fraction; PB2: slowly degradable protein fraction; PC: unavailable protein; TP: true protein; RDP: rumen degradable protein fractions; RUP: rumen undegradable protein fractions; alpha_beta: α-helix to β-sheet peak height ratio; AAI: amide I area; AAI_AII: amide I to amide II peak area ratio; AAII: amide II area; alpha: α-helix peak height; HAII: amide II peak height; HAI_AII: amide I to amide II peak height ratio; RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 level.

Table 5.10. Multiple regression analysis to choose the most important carbohydrates spectral parameters for predicting nutrient profiles, carbohydrate sub-fractions of recently developed faba bean plants.

predicted variable (y)	variable selection ($P < 0.05$)	prediction equation $Y = a + b_1 \times x_1 + b_2 \times x_2 + \cdots + b_n \times x_n$	model R^2	RSD	P value
Basic nutrient profiles (%DM)					
CHO	CEC_AREA, TC1, TC4	CHO = 13.10 CEC_AREA - 96.66 TC1 - 173.36 TC4	0.89	3.19	<0.001
NFC	STC4, CEC_AREA, TC4	NFC = -237.71 STC4 - 10.46 CEC_AREA + 262.92 TC4 + 76.83	0.92	2.63	<0.001
NDF	CEC_AREA, TC4	NDF = 16.41 CEC_AREA - 498.73 TC4 + 30.62	0.86	6.44	<0.001
ADF	CEC_AREA, TC4	ADF = 12.94 CEC_AREA - 452.65 TC4 - 33.05	0.86	5.33	<0.001
ADL	TC3, STC3	ADL = -43.02 STC4 + 80.48 TC3 - 13.17	0.85	1.18	<0.001
Hemicellulose	CEC_AREA	Hemicellulose = 3.38 CEC_AREA - 8.90	0.72	1.73	<0.001
Cellulose	CEC_AREA, TC4	Cellulose = 11.15 CEC_AREA - 341.28 TC4	0.88	4.05	<0.001
Starch	STC1, TC3, TC4	Starch = 228.91 STC1 - 262.90 TC4 + 338.86 TC4 + 22.18	0.93	3.05	<0.001
Carbohydrate sub-fractions (%DM)					
CA4	TC2	CA4 = 44.83 TC2 - 15.59	0.85	0.98	<0.001
CB1	STC1, TC3, TC4	CB1 = 228.91 STC1 -262.90 TC3 + 338.86 TC4 + 23.18	0.93	3.05	<0.001
CB2	CEC_STC	CB2 = 2.44 CEC_STC + 5.33	0.91	1.53	<0.001
CB3	STC3	CB3 = -138.55 STC3 + 49.88	0.32	9.90	0.027
CC	STC4, CEC_AREA, TC1, TC4	CC = 307.33 STC4 + 20.71 CEC_AREA - 129.17 TC1 - 303.36 TC4 - 3.05	0.92	3.82	<0.001

Notes: CHO: carbohydrates; NFC: non-fiber carbohydrate; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; CA4 = sugar (rapidly degradable carbohydrate fraction); CB1 = starch (intermediately degradable carbohydrate fraction); CB2 = soluble fiber (intermediately degradable carbohydrate fraction); CB3 = digestible fiber (available neutral detergent fiber or slowly degradable carbohydrate fraction); CC = indigestible fiber (unavailable neutral detergent fiber); CEC_AREA: cellulosic compounds peak area; TC1: total carbohydrates first peak height; TC4: total carbohydrates fourth peak height; STC4: structural carbohydrates fourth peak height; TC3: total carbohydrates third peak height; STC1: structural carbohydrates first peak height; TC2: total carbohydrates second peak height; RSD: residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 level.

Table 5.11. Multiple regression analysis to choose the most important carbohydrates spectral parameters for predicting estimated energy profiles, rumen degradation parameters of recently developed faba bean plants.

predicted variable (y)	variable selection ($P < 0.05$)	prediction equation $Y = a + b_1 \times x_1 + b_2 \times x_2 + \cdots + b_n \times x_n$	model R^2	RSD	*P* value
Estimated energy values					
tdNFC (%DM)	STC4, CEC_AREA, TC4	tdNFC (%DM) = -201.20 STC4 - 10.45 CEC_AREA + 271.51 TC4	0.92	2.59	<0.001
tdNDF (%DM)	TC1, TC4	tdNDF (%DM) = 75.21 TC1 - 91.96 TC4 - 23.94	0.73	1.96	<0.001
TDN_{1x} (Mcal/kg)	STC3, TC3	TDN_{1x} (Mcal/kg) = 164.23 STC3 - 275.02 TC3 + 124.06	0.85	4.19	<0.001
DE_{1x} (Mcal/kg)	STC3, TC3	DE_{1x} (Mcal/kg) = 8.86 STC3 - 14.17 TC3 + 6.04	0.85	0.22	<0.001
DEp_{3x} (Mcal/kg)	STC3, TC3	DEp_{3x} (Mcal/kg) = 7.26 STC3 - 11.10 TC3 + 5.09	0.85	0.17	<0.001
ME_dairy (Mcal/kg)	STC3, TC3	ME_dairy (Mcal/kg) = 8.97 STC3 - 14.63 TC3 + 5.65	0.85	0.22	<0.001
NE_{L_3X} (Mcal/kg)	STC3, TC3	$NEL_{\_3X}$ (Mcal/kg) = 4.03 STC3 - 6.72 TC3 + 3.04	0.84	0.10	<0.001
ME_beef (Mcal/kg)	STC3, TC3	ME_beef (Mcal/kg) = 7.27 STC3 - 11.88 TC3 + 4.96	0.85	0.18	<0.001
NE_m (Mcal/kg)	STC3, TC3	NEm (Mcal/kg) = 6.64 STC3 - 10.67 TC3 + 3.79	0.85	0.16	<0.001
NE_g (Mcal/kg)	STC3, TC3	NEg (Mcal/kg) = 6.02 STC3 - 9.60 TC3 + 2.95	0.85	0.15	<0.001
Rumen degradation parameters					
D (%)	CEC_AREA	D (%) = -10.03 CEC_AREA + 668.11 TC4 + 8.93	0.69	9.44	<0.001
EDNDF (%)	STC1, STC3	EDNDF (%) = -289.54 STC1 + 215.88 STC3 + 21.97	0.68	6.62	0.001

RSD: tdNFC: truly digestible non-fiber carbohydrates; tdNDF: truly digestible neutral detergent fiber; TDN1×: total digestible nutrient at one time maintenance. $DE_{1\times}$; digestible energy at production level of intake (1×); $DE_{p3\times}$: digestible energy at production level of intake (3×); ME: metabolizable energy at production level of intake; $NE_{L3\times}$: net energy for lactation at production level of intake (3×); NEm: net energy for maintenance; NE_g: net energy for growth; D: potential degradable fraction in the in situ incubation; EDNDF: effective degraded neutral detergent fiber; STC4: structural carbohydrates fourth peak height; CEC_AREA: cellulosic compounds peak area; TC4: total carbohydrates fourth peak height; STC3: structural carbohydrates third peak height; TC3: total carbohydrates third peak height; STC1: structural carbohydrates first peak height; residual standard deviation; R^2: coefficient of determination. All variables left in the final model were significant at the 0.05 level.

5.7. Chapter conclusions

Protein related spectral parameters are consists of those related to specific chemical structure of protein (peaks of amide I and amide II) and those related to secondary structure of protein (α-helix and β-sheet). Peak of amide I is associated with stretching vibration of the C=O bond of the amide and absorption of peak in amide II area is associated with bending vibration of the N-H bond. Metabolizable protein supply and estimated intestinal protein digestion of faba bean samples had stronger correlation with spectral profiles in original samples than in incubation residue samples for both secondary structures related spectral profiles and chemical structure related spectral profiles. As a result, the digestive characteristics of protein is mainly determined by its original spectral profiles. Effective degradable protein was found to have strong correlation with amide I to amide II peak height ratio and ratio of α-helix to β-sheet, which is consistent with previous studies that protein with higher proportion of α-helix is of higher quality. For basic protein fractions, 24 h incubation residue spectral profiles were found to have strong negative correlation with NDICP content of original samples. The possible explanation for this is that with higher NDICP content in samples, higher proportion of NDICP was left after rumen degradation and the residue of rumen degradation is of low quality thus having low α-helix to β-sheet ratio. More studies are needed to unveil the alteration of molecular structure by ruminal microbial digestion and the consequent change of nutrition availability.

In term of the results of correlation study for carbohydrate related profiles, spectral parameters of incubation residue samples were found to have stronger correlation with less degradable carbohydrate fractions and degradation parameters while original samples spectral profiles have stronger correlation with easily degradable carbohydrate fractions. Specifically, starch had strong negative correlation ($r = -0.92$) with 2nd peak height of structural carbohydrate (around ca.1372

cm^{-1}) and strong negative correlation (r = -0.95) with 3rd peak height of total carbohydrate (around ca.1103 cm^{-1}) in original samples spectral profiles. Intermediate digestible carbohydrate fraction hemicellulose was found to have strong negative correlation with peak area of structural carbohydrate region (ca.1186-1486 cm^{-1}) with correlation coefficient equals -0.84 in 12 h degradation residue spectral profiles. Additionally, undigestible carbohydrate fraction ADF and ADL was found to have strong negative correlation with 1st peak height of structural carbohydrate (ca.1322 cm^{-1}) with correlation coefficient of -0.84 and -0.85 respectively. As a result, rumen degradation of carbohydrate contents can be reflected in the change of its spectral profiles.

For results of multi-linear regression, carbohydrate and protein related spectral features could be used as indicators for faba bean nutritional evaluation in dairy cattle.

Overall, rumen degradation of carbohydrate contents can be reflected in the change of its spectral profiles which may because the degradation of feed particle lead to the break down of specific chemical bonds thus leading to change of spectral features; protein availability and digestibility are mainly associated with original sample spectral profiles which may because the release of NDICP during rumen degradation account for small part of total digestible CP fractions. In addition, NDICP was associated with incubation residues samples may because the cross-linkage between NDICP and cell wall fraction interfere the absorption of peptide bonds in mid-infrared region and the ruminal degradation cleaves the cross-linkage between NDICP and cell wall fractions and makes it detectable by FTIR. Moreover, carbohydrate and protein related spectral features could be used as indicators for faba bean nutritional evaluation in dairy cattle.

To answer the question how the data from spectra should be utilize to daily production, two aspects should be clarified. Firstly, what sample should be used. When predicting nutritional values of different faba bean samples, the general equation established by including different samples could

be used. However, if you need to get more precise prediction, equation could be built by using exact sample you want to use in diet formulation. For example, by including faba bean leaf, stem, whole pods, whole plant and whole plant silage, one equation suitable for all faba bean samples could be acquired, which is applicable for all faba bean samples but with less precision. But when only faba bean silage is what you want to include in your diet, you just need to use the spectral profiles from faba bean silage to establish prediction equation. Moreover, instead of multi-linear regression, partial least square regression model could be used with larger sample sizes. Nevertheless, the nutritional and spectral feature of faba bean partitions could be used as reference for plant breeding. For example, the unbalanced nitrogen and energy supply during digestion was found in faba bean whole pods and the NDF content was found to be significantly higher in stem. In addition, the protein content of faba bean seed was found to be heritable (Khazaei and Vandenberg 2020). Therefore, when considering breeding faba bean to be used as forage source for ruminant, the trait of NDF content and readily digestible contents of different faba bean partition could be used as an indicator.

The second question is if the original sample or degradation residue sample spectral profiles should be used for nutritional and digestion values prediction. When answering this question, we should notice that the biology nature of feed relating to its nutritional features is correlated with spectral change during digestion. Therefore, by including the degradation residue sample spectral profiles, more precise prediction could be acquired. However, in practical production, large number of samples are needed to be incubated, thus needing large amount of fistulated cattle, which involves error. However, as for some specific parameters such as readily carbohydrate fraction, metabolizable protein, predicted effective degradable crude protein, original sample spectral profiles should be enough. In addition to the application of spectra feature on feed evaluation, its

possibility to improve our understand of microbial digestion should also be considered, as the spectra feature change involves not only digestion of chemical contents but also cleave of chemical bonds. Therefore, from the perspective of author, faba bean spectral change during ruminal digestion is more valuable for scientific purpose.

6. RESEARCH DISCUSSION AND CONCLUSION

The growing population of world urge us to make the most reasonable use of our land. To achieve this goal, converting the energy and protein sources which human cannot utilize to high quality animal protein is one of the best choices. Western Canada has its advantage in plenteous land resources suitable for plantation of legumes and crops. The most commonly used legume sources are dry beans, dry peas, lentils and chickpeas. Faba bean as legume source with seeds containing high crude protein and starch contents has been fed to livestock and acquire good results. In addition, faba bean is the legume with the highest N-fixing ability, thus optimal for crop rotation. The tannin content in faba bean seed is major concern for feeding faba bean to livestock, however, the moderate tannin content slows down the protein releasing rate and benefits for N utilization in ruminant. As a result, faba bean could be considered as a perfect feeding choice for ruminant in Canada. There are only limited studies about the feeding values of faba bean to dairy cattle and most of them focus on feeding value of faba bean seeds. Recent study has found that feeding faba bean silage to high production dairy cattle can acquire considerate revenue. However, research about feeding value of different partition of faba bean cannot be found. As a conclusion, this study was conducted for determining the feeding values of faba bean samples (whole plant, stem, leaf, whole pods and whole plant silage) and using different faba bean samples as reference to study the effect of ruminal degradation to the change of spectral profiles and how the change is associated with nutritional utilization and availability.

Chapter 3 of this study was to determine the nutritional values of different faba bean partition samples in terms of chemical and nutrient profiles, energy profiles, CNCPS fractions, rumen degradation kinetics, N to energy synchronization, estimated intestinal digestibility of rumen undegradable protein and modelling of truly absorbable protein to dairy cattle using NRC-2001

and DVE/OEB systems. The study to compare the nutritional and digestion characteristics of partitioning of faba bean or other legumes cannot be found. According to the results of this study, significantly different nutritional values of faba bean samples ($P < 0.001$) was observed. Faba bean whole pods had the highest crude protein with 74% of which was soluble crude protein and NDICP and ADICP only account for 5% of total crude protein. As a result, whole pods faba bean was a great source of protein for ruminant. Starch was another nutrient composition for whole plant faba bean which was 261 g/kg DM compared with 215 g/kg DM of NDF. ADF and ADL in whole pods of faba bean were also the lowest among the faba bean samples, which were 156 and 15 g/kg DM respectively. Whole plant silage compared with whole plant faba bean had higher crude protein content 212 versus 187 g/kg DM with soluble crude protein comparable between each other. Starch was also higher in whole plant faba silage. NDF and ADF contents in whole plant and whole plant silage is comparable with each other. Leaf of faba bean only accounted for 6.1% of the total weight of whole plant. Faba bean leaf had crude protein content similar with whole plant and lower soluble crude protein but had the highest ether extract contents. Stem of faba bean accounted for 27.4% of total weight and is of lowest nutritional value with the lowest crude protein and the highest NDF and ADF. Th energy profiles gave similar conclusion as chemical profiles with whole pods of faba bean having the highest tdNFC, tdCP and the lowest tdNDF thus having the highest TDN. Faba bean silage had higher tdCP than whole plant faba bean but the energy values (total digestible nutrient, digestible energy, metabolizable energy and net energy) of faba bean silage were only numerically higher than faba bean whole plant. Although faba bean whole plant and faba bean silage had energy value for dairy cattle, it needs to be noticed that the relatively higher proportion of readily fermentable carbohydrate fractions (starch and sugars) and readily fermentable protein fraction (soluble crude protein) may cause nutritional disorder for high production dairy cattle

(acidosis and bloat). As a result, although whole plant, whole pods and whole plant faba bean silage had high nutritional values and could be used as feeding resources for dairy cattle, it should cooperate with roughage to prevent the nutritional disorder caused by rapid fermentation.

Regarding feed degradation kinetics in the rumen, 81% of the total NDF was degradable in whole pods, which was the highest among the faba bean samples. Effective degradable NDF was also the highest in WP. Degradable fraction of NDF in whole plant silage was numerically higher than whole plant. However, the EDNDF was higher in WPL than whole plant silage, which was because the greater degradation rate of WPL compared with faba silage (8.59 % vs 4.41%). As for OM degradation kinetics, degradation rate was similar for WPL, WP and whole plant silage. However, EDOM was significantly different, with WP the highest, followed by whole plant then the faba silage.

In order to maximize feed efficiency and to maintain animal health during production, moderate and synchronized fermentation of carbohydrate and N source are important. For health concern, rapid fermentation of feed is major induce of acidosis and bloat and for production concern, avoid rapid fermentation can increase the microbial protein production and reduce waste of N in form of urea. According to the publications (Sinclair et al. 1993; Tamminga et al. 1994), optimal N to energy synchronization could be achieved when 25 g of available N per kilogram of OM fermented in the rumen or 32 g of available N per kilogram of available CHO. According to the results of this study, very unbalanced protein and carbohydrate fermentation pattern was seen, especially for faba silage and whole pods faba bean. The N is rapidly degraded in the first 5 hours, which may cause adverse effects to cattle. As a result, providing more roughage in the feed to dilute the fermentation rate is recommended when choosing to feed whole plant faba bean or faba silage.

The intestinal digestibility of rumen undegradable protein is also predicted and WP had the highest intestinal protein digestibility (87.24%) which was 12% higher than WPL and 31% higher than faba silage.

Truly absorbable nutrient was predicted with both NRC-2001 and DVE/0EB systems. Slightly different results were obtained with these two systems. For DVE/OEB system, WP was predicted to have the highest truly digestible protein in small intestine (97 g/kg DM). WPL was predicted to have the second highest DVE value (78 g/kg DM), which was 18 % higher than faba silage. Leaf of faba bean was numerically slightly lower than faba silage but still eight times greater than that of stem. Comparatively, metabolizable protein supply was predicted to have small difference among faba bean samples by NRC-2001 system. Specifically, WPL, WP and faba silage were predicted to have similar MP supply, which were 81, 87 and 80 g/kg DM respectively. Leaf of faba bean was predicted to provide 71 g/kg DM of MP and was 2.4 times greater than stem of faba bean. As for feed milk value, the same trend was observed for these two systems, Overall, in both systems, WP, WPL and faba silage were predicted to have comparable truly absorbable protein supply with alfalfa and was higher than timothy according to the data of Yu et al (2003). As a result, faba bean whole plant, whole pods and faba silage have potential to be used as alternative feed for dairy cattle.

The traditional wet chemistry analysis is to use combination of specific chemical reactions to quantify a group of compounds with similar chemical and nutritional property. However, we should notice that the digestion progress in the rumen is achieved by a series of enzymatic hydrolysis of specific chemical bonds which cannot be revealed by wet chemistry. FTIR instead is using the unique absorption of mid-infrared light at different frequencies. Thus, it reveals more information about change of specific chemical bonds and may provide more useful information

when taking spectral change into consideration. Protein secondary structure is one factor that affecting the digestibility of feed and FTIR spectroscopy could be used to detect the proportion of protein secondary structure compositions (Duodu et al. 2003; Yu 2005b). In addition, plant cell wall complex in not uniform in terms of chemical, physical or nutritional characteristics (Van Soest et al. 1991), thus FTIR is favorable to study the spectral structural change of plant cell wall during rumen degradation. In conclusion, the Chapter 4 of this study was conducted to determine the change of carbohydrate and protein related structural spectral features during rumen incubation.

According to the results of univariate analyses of protein related spectral features, spectral intensity of β-sheet and amide I peak height, amide region and amide II peak area and peak area ratio of amide I to amide II were significantly reduced after first 12 h or rumen degradation, however spectral intensity was not significantly changed during the second 12 h of rumen degradation. For spectral intensity of amide II and α-helix peak height, amide peak area and peak height ratio of amide I to amide II and α-helix to β-sheet, incubation time was significantly interacted in different faba bean samples. The results of this study partly agree with the results of Xin and Yu (2013b), where spectral intensity of all protein related parameters were reduced after rumen incubation except for amide II area. In addition, amide I peak was found to decrease linearly with the increase of incubation time. For spectral ratio of amide I to amide II peak height, stem was found to increase dramatically with incubation time increase while other faba bean samples only increased slightly. According to the original spectra, increase of amide I peak height and minimal change of amide II peak height were observed for stem. For spectral intensity of α-helix to β-sheet ratio complicated patterns were founded for different faba bean samples. Regarding carbohydrate related spectral profiles, faba bean samples were significantly interacted with incubation time for all spectral

parameters except first peak height of total carbohydrate (around ca. 1024 cm^{-1}) and peak height of cellulosic compound (around ca. 1240 cm^{-1}). For those two spectral parameters, significant increase after first 12 h of rumen incubation was observed.

The multivariate analysis conducted in amide region (ca. 1486-1715 cm^{-1}), total carbohydrate region (ca. 938-1186 cm^{-1}) and structural carbohydrate region (ca. 1186-1486 cm^{-1}) and consistent results were acquired which shows rumen degradation to the change of spectral profiles happened most first 12 h of rumen incubation. The results demonstrate dramatically change of spectral chemical feature of feed during rumen degradation and could be used as an indicator for feed degradation in the rumen.

As a conclusion, during rumen degradation, chemical spectral features were intensively changed and were differentiated according to different partition samples, which reflect the hydrolysis of same chemical bonds were interacted with different chemical compositions. In depth spectral analysis should be conducted to give detailed explanation of specific chemical bonds hydrolysis during rumen incubation.

To associate the spectral feature change to nutrient availability and digestibility, correlation and regression studies were performed (Chapter 5). As for truly absorbable protein profiles, DVE/OEB system compared with NRC-2001 system had higher correlation with original sample spectral profiles. For DVE/OEB system, DVE value was highly corrected with α-helix peak height ($r = 0.87$) and α-helix to β-sheet peak height ratio ($r = 0.85$), which consistent with the conclusion that higher α-helix ratio in the protein composition is correlated with higher protein quality (Yu 2005b). Rumen undegradable protein intestinal digestibility was also found to highly correlated with α-helix to β-sheet peak height ratio ($r = 0.85$), however, the results disagree with the study of Peng et al. (2014). The possible explanation of the different results were different sample types, thus

proving protein secondary structure only account for partial reason of total digestibility and utilization (Duodu et al. 2003). The protein degradation kinetics parameters (k_d, S, D) were found have no correlation with spectral profiles. Hence spectral structure characteristics play limited role in determining protein degradation rate in the rumen and physical property of feed and special linkage between protein and cell wall structure may play more important role in degradation rate. CP and SCP were more correlated with original sample spectral profiles as large proportion of CP and SCP were readily fermented in the rumen. Undigestible ADICP was found to have strong negative correlation with amide I to amide II peak height ratio of original samples (r = -0.73). NDICP was found to have strong negative correlation with α-helix to β-sheet peak height ratio in 24 h incubation residue samples (r = -0.82). The possible reason could be with the degradation of plant cell wall complex, the interfere of the chemical bond between the protein (NDICP) and carbohydrate was cleaved, after that the secondary structure of NDICP was then detected.

The correlation between carbohydrate related spectral profiles and carbohydrate compositions was found to related to carbohydrate digestion characteristics. The carbohydrate fractions that are more easily digested in the rumen had stronger correlation with original samples spectral profiles. Specifically, starch was found to have strong negative correlation with 2nd peak height of structural carbohydrate region (r = -0.92) and 3rd peak height of total carbohydrate region (r = -0.95); hemicellulose had strong negative correlation with structural carbohydrate area (r = -0.84) and 3rd peak height of structural carbohydrate region (r = -0.80) of 12 h incubation samples spectral profiles. Less digestible carbohydrate fractions ADF, ADL and ADL had stronger correlation with 24 h incubation residue samples spectral profiles. The possible explanation was the different chemical bond of different polysaccharide, for example α 1,4 linkage of glucose in starch, β 1,4 linkage of glucose of cellulose and bonds connecting glucose, mannose, galactose, xylose and

arabinose in hemicellulose. All these different bonds were hydrolyzed at different rate in the rumen therefore polysaccharides with different bonds were associated with spectral profiles with different degradation duration. As a result, spectral feature change during rumen degradation provides information about chemical structure features change during degradation and could be used as an indicator to assist nutritional research about polysaccharide degradation. Regression study found nutritional parameters of DVE, FMV and EDCP. CNCPS parameters of CB1, CB2 and CC fractions and tdNFC carbohydrate were predicted with regression coefficient greater than 0.90. Therefore, spectral features could be used to predict protein quality and different carbohydrate fractions of feed.

Overall, different faba bean partition samples had different nutritional quality, faba bean whole plant, whole pods and whole plant silage were all found to have potential to be used as alternative feed resources for ruminant. In addition, spectral features were found to change dramatically during the ruminal fermentation and the structural spectral features change during degradation were associated with unique nutritional and digestion features of feed and could be used to predict nutritional and digestion characteristics of feed.

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